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Ubiquitin E3 ligase UHRF1 regulates p53 ubiquitination and p53-dependent cell apoptosis in clear cell Renal Cell Carcinoma

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ABSTRACT

Ubiquitin-like with PHD and RING finger domain 1 (UHRF1) is a multi-domain ubiquitin E3 ligase that plays critical roles in regulation of DNA methylation and histone ubiquitination. In this study, we found UHRF1 is frequently overexpressed in human clear cell Renal Cell Carcinoma (ccRCC) tissues both at mRNA and protein levels. We showed that UHRF1 directly interacts with p53 both *in vivo* and *in vitro*. A new domain (PD) in UHRF1 was required for interaction with p53. We found that UHRF1 down-regulates p53 transactivation activity which was depends on the ubiquitin E3 ligase function. UHRF1 can promote non-degradative ubiquitination of p53, suppress p53 pathway activation and p53-dependent apoptosis in ccRCC cells. Together, our study suggests that UHRF1, which overexpressed ccRCC, may act as a p53 regulator, suppress p53 pathway activation and help ccRCC cells to escape from p53-dependent apoptosis.

1. Introduction

Kidney tumor is one of the most common causes of cancer death, which estimated to be diagnosed in more than 270,000 individuals every year worldwide [1]. The majority of malignant kidney tumors are Renal Cell Carcinomas (RCC), of which approximately 70% are clear-cell type (ccRCC) [2]. Although many signaling pathways have been implicated in ccRCC, the molecular basis of ccRCC carcinogenesis remains largely unknown. For effective cancer prevention and treatment, it is necessary to investigate the molecular pathogenesis of ccRCC.

Ubiquitin-like with PHD and RING finger domain 1 (UHRF1) is modular with multiple domains, including N-terminal ubiquitin-like domain (NIRF), the Plant Homodomain (PHD), the Set and Ring Associated domain (SRA) and RING domain. The C- terminal RING domain of UHRF1 confers intrinsic E3 ligase activity toward histones and non-histone proteins, such as PML and DNMT1 [3–5]. UHRF1 was reported acting a crucial role in DNA methylation by recruiting DNMT1 to hemimethylated DNA during DNA replication [6,7]. UHRF1 contributes to DNA damage repair as a lesion recognition factor and nuclease scaffold [8]. UHRF1 was reported to have a high expression level in proliferating tissues and low expression in quiescent cells and tissues [9]. UHRF1 is involved in cell cycle regulation by promoting G1/S transition [10,11]. Indeed, UHRF1 was reported up-regulated in various human cancers [12]. Knockdown of UHRF1 expression in cancer cells significantly suppressed cell growth, indicating that UHRF1 was essential for progression of cancers [13]. Collectively, UHRF1 may serve as an attractive biomarker and therapeutic target for cancer treatment [3,14,15].

The p53 tumor suppressor plays important roles in maintenance of genome stability and protection against malignant transformation [16]. p53 mutations have been documented in more than half of all human cancers [17]. The cellular functions of p53 are rapidly activated in response to various stresses to cause cell cycle arrest and apoptosis [16]. p53 stability is tightly regulated by multiple signaling pathways through distinct mechanisms, in which Mdm2 acts as a primary factor that binds to p53 and functions as an ubiquitin E3 ligase to promote p53 ubiquitin-dependent degradation by the proteasome [16]. In addition to mdm2, other ubiquitin E3 ligases such as COP1, Pirh2, and ARF-BP1 had been

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discovered to regulate p53 ubiquitination and degradation in the certain context [18].

In the present study, we demonstrated that the UHRF1 is frequently overexpressed in human ccRCC tissues. Moreover, we demonstrated UHRF1 directly interacts with p53, and promote p53 non-degradative ubiquitination. Lastly, we showed that UHRF1 suppresses p53-mediated transactivation and apoptosis in response to DNA damage signal, which may partly explain the tumor-promoting functions of UHRF1.

2. Materials and methods

2.1. Cell culture and transfection

The 293T, Caki-1 cells were obtained from American Type Culture Collection (ATCC). 293T cells were maintained in DMEM with 10% (vol/vol) fetal bovine serum (FBS), Caki-1 cells were maintained in McCoy's 5A medium with 10% FBS, at 37 °C in a humidified incubator with 5% CO_2. Cells were transiently transfected using Lipofectamine 2000 or 3000 (Invitrogen) according to manufacturer's instructions.

2.2. Human tissue samples

ccRCC tissues of surgical resection specimens were obtained from Shanghai First People's Hospital. The tumor specimens were stored at −80 °C for further analysis. Study was done with the approval of the hospital Ethics Committee. Informed consent was obtained from each subject or subject's guardian.

2.3. Expression constructs

Human UHRF1 cDNAs were amplified from Human liver Marathon cDNA library (Clontech), and subcloned into pGEX-4T-2, pCIN4-FLAG–HA, and pCMV-Myc expression vectors. The deletion and point mutant constructs of UHRF1 or p53 were generated by the KOD-Plus Mutagenesis Kit (TOYOBO).

2.4. RNA interference

The siRNA oligos were purchased from Genepharma Inc. The siRNA oligos sequences for UHRF1 are: si-UHRF1#1: 5′-GCUCAUGUGCGAUGAGUGC-3′; si-UHRF1#2: 5′-GGUCAAUGAGUACGUCGAU-3′. The siRNA oligos sequences for p53 are: si-p53: 5′-TGCGTGTGGAGTATTTGGATG-3′. The sequence of negative control is: si-Control: 5′-ACAGACUUCGGAGUACCUG-3′.

2.5. Antibodies

UHRF1 (A301-470; Bethyl lab), p53 (Do-1; sc-126; Santa Cruz); acetyl-p53 (Lys382) (2525S; Cell Signaling), phospho-p53(Ser15) (9286S; Cell Signaling), p21 (3733-1; epitomics); Myc (9E10; Sigma), Flag (M2; Sigma), HA (MM5-101R; Millipore) and Actin (AC-74; Sigma).

2.6. GST pulldown assay

293T cells were lysed with 1 × cell lysis buffer at 4 °C after 24hr transfection. GST fusion proteins were immobilized on glutathione-Sepharose beads (Amersham Biosciences), which were incubated with lysates of 293T cells or *Escherichia coli* expressed and purified recombinant His-p53 protein for 2 h, after washing with pull-down buffer. Another five times wash with binding buffer, the beads were resuspended in sample buffer. The bound proteins were analyzed by SDS-PAGE.

2.7. Immunofluorescence

Cells were cultured and transiently transfected on coverslips, which were blocked with 10% normal horse serum plus 1% BSA for 1 h, after fixed in 4% paraformaldehyde for 10 min and permeabilized in 0.2% Triton X-100 for15 min at room temperature, the treated cells on the coverslips were incubated overnight at 4 °C using mouse anti-UHRF1antibody (1:200 dilution). The cells were incubated with CF555 Donkey Anti-Rabbit secondary antibody (1:300 dilution) for 1 h and stained with DAPI, after being washed three times in TBS containing 0.1% Tween 20. The fluorescent images were captured by Olympus Inverted Microscope System.

2.8. Quantitative RT-PCR

Total RNA was isolated from transiently transfected cells using the TRIzol reagent (Tiangen), and cDNA was reversed-transcribed using the Superscript RT kit (TOYOBO), according to the manufacturer's instructions. PCR amplification was performed using the SYBR Green PCR master mix Kit (TOYOBO). The Primers of UHRF1 are: UHRF1-F: 5′-GCAGAGGCTGTTCTACAGGG-3′, UHRF1-R: 5′-GTGTCGGAGAGCTCGGAGT-3′. All quantization were normalized to the level of endogenous control GAPDH.

2.9. Apoptotic assay

Caki-1 cells were seeded overnight in 6-well plates. 48hr after transfection, cells were treated with 20 μM 5-FU for 24 h. The cells were collected and washed with PBS and incubated in PBS containing100 μg/ml RNase A, 0.03% TritonX-100, and 50 μg/ml propidium iodide (PI) for 15 min at room temperature. DNA content and cell cycle were assessed by FACScan based on PI staining. Cells in sub-G1 were considered apoptotic.

2.10. Immunohistochemical staining and image analysis

The tissue microarray sections were deparaffined in xylene and rehydrated in alcohol. Endogenous peroxidase activity was blocked by 3% H_2O_2 for 10 min. After twice heating by microwave in a citrate acid buffer (pH 6.0), the Antigen retrieval was achieved, and sections were blocked in 3%goat serum (Boster Bio) at 37 °C for 15 min. Slices were incubated with a primary antibody (anti-UHRF1 at 1:60 dilution) at 4 °C overnight, followed by three washes in PBS and incubation with a HRP-labeled secondary antibody (goat anti-rabbit IgG at 1:100 dilution) for 30 min. Immunostaining was achieved using 3,3′-diaminobenzidine tetra hydrochloride. All slides were counterstained with hematoxylin. Images were obtained by a Leica DMRI microscope installed on a Lexica DC500 camera (Leica).

2.11. Statistical analysis

The data in this study were expressed as the mean ± S.D. from three independent experiments. The levels of UHRF1 between the normal and RCC tissues were compared by Wilcoxon signed rank sum test and X^2 test. Student's t-test was used to evaluate the significance of inter-group differences. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. UHRF1 was overexpressed in ccRCC tissues

To explore whether the expression level of UHRF1 was altered in human ccRCCs, we first examined the mRNA level of UHRF1 in 30

different human ccRCC tissues by quantitative RT-PCR methods. As shown in Fig. 1A, the mRNA expression of UHRF1 was significantly elevated in 21 of 30 (70%) cases compared with adjacent non-tumor tissues. 3 of 30 (10%) cases showed no significant alteration, and only 6 of 30 (20%) cases showed slight down-regulation of UHRF1 in ccRCCs. This result revealed UHRF1 expression was elevated in a large proportion of ccRCC tissues (Fig. 1A, $p < 0.001$). In agreement with the increased expression of UHRF1 mRNA in ccRCCs, UHRF1 protein expression was also up-regulated in 18 out of 23 ccRCCs as assessed by western blot (Fig. 1B). Moreover, a tissue microarray containing 31 paired ccRCCs and adjacent non-tumor tissues was immunostained using anti-UHRF1 antibody. Substantial UHRF1 immunostaining was detected in the ccRCC samples, whereas moderate UHRF1 staining was observed in non-tumor samples (Fig. 1C). Collectively, we demonstrated by various approaches that UHRF1 mRNA and protein are frequently overexpressed in human ccRCC tissues.

3.2. UHRF1 interacts with p53 both in vivo and in vitro

Previous studies had mentioned that the tumor suppressor p53 indirectly downregulates UHRF1 through the up-regulation of p21/WAF1 and subsequent deactivation of E2F1 [10]. We want to investigate whether UHRF1 negatively regulates p53 activity in a feedback loop manner. We first sought to confirm whether UHRF1 and p53 proteins interact with each other. We co-expressed Myc-p53 and FH (Flag-HA)-UHRF1 plasmids in 293T cells and immunoprecipitated UHRF1 using anti-Flag antibody. As shown in Fig. 2A, FH-UHRF1 co-immunoprecipitated with Myc-p53. Moreover, endogenous p53 was also detected in the immunoprecipitates when endogenous UHRF1 was immunoprecipitated from Caki-1 cells by anti-UHRF1 antibody (Fig. 2B). To further examine the interaction between UHRF1 and p53 *in vivo*, we determined whether these two proteins are localized to the same subcellular compartments. As shown in Fig. 2C, when GFP-p53 and were transiently expressed in Caki-1 cells, GFP-p53 and endogenous UHRF1 were exclusively co-localized in nucleus. To determine whether the interaction between p53 and UHRF1 is direct, we assessed their interaction *in vitro* using purified recombinant proteins. Recombinant His-p53 and GST-UHRF1 were expressed and purified from bacteria. As shown in Fig. 2D, GST-UHRF1, but not GST alone, bound to His-p53 in a pulldown assay.

Next, we want to delineate the domains mediating the mutual interactions between UHRF1 and p53. UHRF1 is modular with multiple domains, including NIRF, PHD, SRA, and RING domain. To determine which domain mediates its interaction with p53, we generated a series of truncation mutants of UHRF1 (Fig. 2E, right panel). A total of ten mutants were generated as GST fusion proteins and expressed and purified from bacteria. A pulldown assay with GST-UHRF1 and the various truncation mutants was used to assess their interactions with Myc-p53 overexpressed in 293T cells. As shown in Fig. 2E (left panel), we found that the p53-binding domain (PD) of UHRF1 was not localized to any known domains, whereas it was localized a region (600–700aa) between the SRA and RING domain. A similar strategy was used to map the UHRF1-binding domain of p53. We generated a series of mutants of p53 and determined the minimal region that mediates its interaction with UHRF1. As shown in Fig. 2F, the C-terminal regulatory domain of p53 is sufficient for UHRF1 binding. Taken together, these results indicate that UHRF1 interacts with p53 both *in vitro* and *in vivo*.

3.3. UHRF1 promotes non-degradative ubiquitination of p53

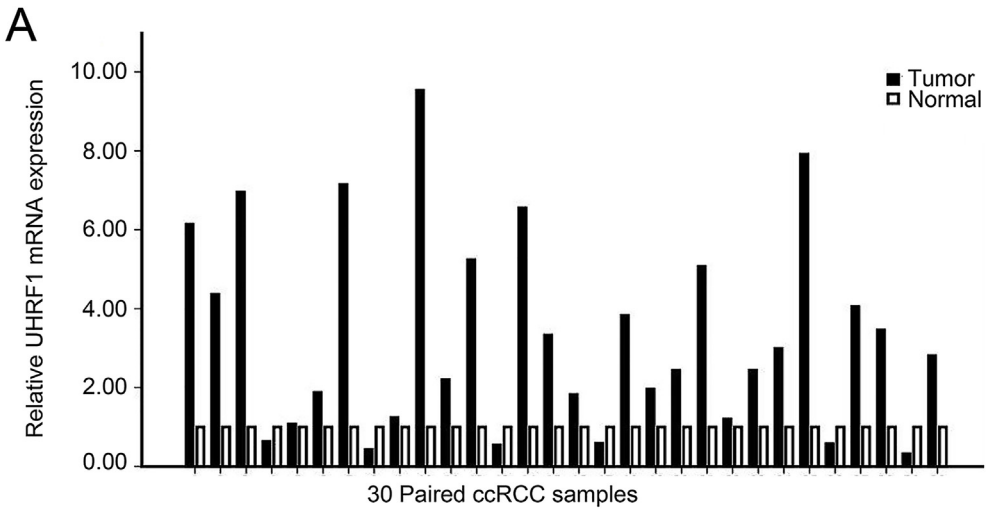
UHRF1 is an RING domain-containing ubiquitin E3 ligase, which was reported to target various substrates, such as PML or DNMT1, for ubiquitin-dependent degradation [4,5]. So we want to investigate whether UHRF1 like Mdm2, negatively regulates p53 turnover through ubiquitin-dependent degradation. First, we examined whether UHRF1 can ubiquitinate p53 *in vitro*. As shown in Fig. 3A, when recombinant p53 and UHRF1 or Mdm2 (positive control) were incubated in ubiquitin reaction buffer that contained recombinant E1, E2 (UbcH5a), and His-ubiquitin, strong ploy-ubiquitinated bands of p53 were detected by Western blot, suggesting UHRF1 can promote p53 ubiquitination *in vitro* like Mdm2. Next, we investigated which domain of UHRF1 was indispensible for p53 ubiquitination. We constructed a UHRF1 mutant (UHRF1-C724A), in which the amino acids Cys (C724) were mutated to Ala (A). Cys724 is a conservative amino acids localized in the RING domain, of which the mutation led to an inactivation of intrinsic ubiquitin E3 ligase. As expected, the UHRF1-N3 mutant that does not contain RING domain or UHRF1-C724A mutant totally lost the ability to ubiquitinate p53 *in vitro* (Fig. 3B), suggesting that UHRF1 promotes p53 ubiquitination directly via its E3 ligase activity conferred by the RING domain. The SRA domain of UHRF1 is critical for binding to hemimethylated CG sites [5,6]. However, SRA deletion mutants (UHRF1-ΔSRA) can still ubiquitinate p53 *in vitro*. In contrast, the p53-binding domain deletion mutant (UHRF1-ΔPD) totally lost the ability to ubiquitinate p53 *in vitro* (Fig. 3A). Taken together, these results suggest that both the RING domain and the PD domain were required for the UHRF1-mediated p53 ubiquitination. Moreover, a cell-based ubiquitination assay was performed to prove that UHRF1 can promote p53 ubiquitination *in vivo* (Fig. 3C).

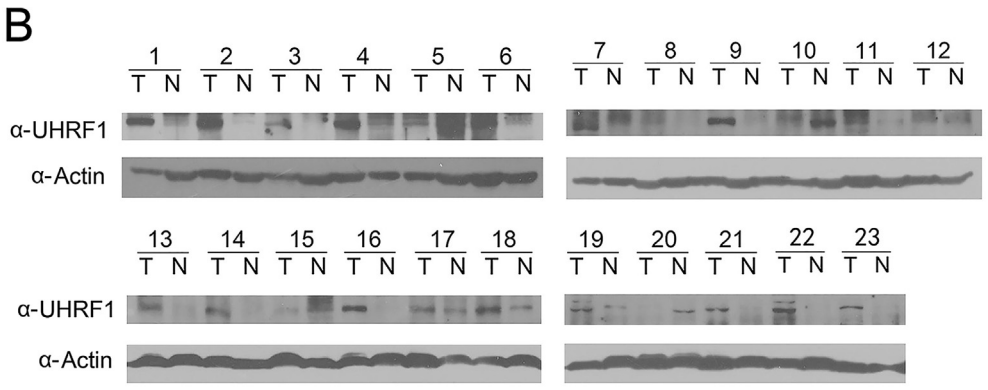
Since poly-ubiquitination most frequently targets its substrate for degradation, we then sought to test whether UHRF1 can promote p53 degradation like Mdm2. A co-transfection experiment in 293T revealed that exogenous p53 protein levels remain unchanged in the presence of increasing levels of UHRF1 (Fig. 3D). To confirm that UHRF1 does not regulate p53 stability, we further assessed endogenous p53 protein levels in Caki-1 cells (p53-wildtype ccRCC cells) following knockdown of UHRF1. As shown in Fig. 3E, Endogenous UHRF1 proteins in Caki-1 cells were severely depleted after transfection with two independent siRNAs, however, p53 protein level was unaffected. Taken together, these data suggests that UHRF1 promotes p53 non-degradative ubiquitination, but does not affect p53 stability.

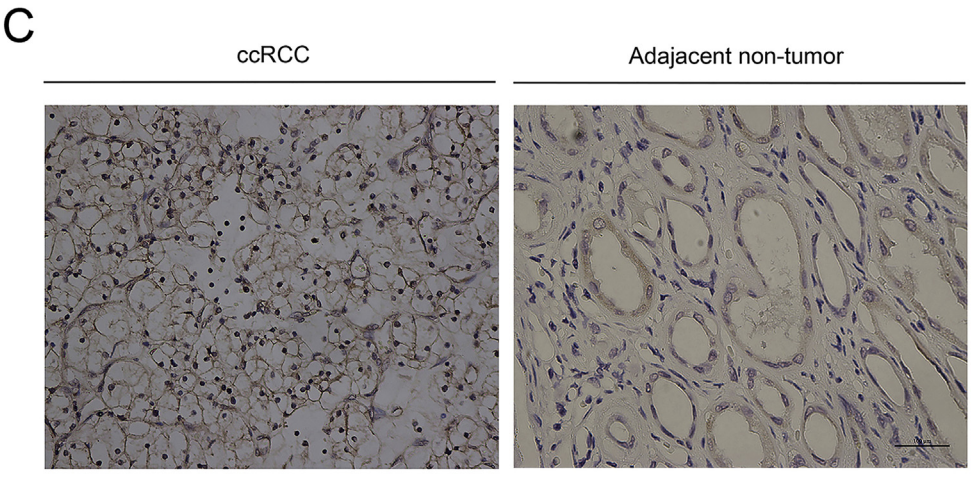
3.4. UHRF1 suppresses p53-dependent transactivation and apoptosis

Since we demonstrated that UHRF1 interacts with p53, we want to further explore the potential functional relationship between UHRF1 and p53. To this end, reporter gene assays was performed to determine whether UHRF1 affected the transcriptional activity of p53. The p53 responsive element reporter (p53-Luc) was co-transfected with an increasing amount of UHRF1 plasmids. As shown in Fig. 4A, the luminescence outputs showed that the p53-luciferase reporter activity significantly decreased with the increasing amounts of UHRF1-WT. To further confirm that UHRF1-mediated suppression of p53 reporter activity was dependent on its ubiquitin E3 ligase activity, we repeated the reporter assay using UHRF1-C724A mutant. As shown in Fig. 4A, co-expression of UHRF1-C724A mutant had no effect on p53 reporter activity. These results suggest that UHRF1 down-regulates p53 transactivation activity, which is dependent on the RING domain, which confers ubiquitin E3 ligase activity.

Given that DNA damage is one of the major stresses that provoke p53 action, we examined the p53 protein stabilization in UHRF1-depleted Caki-1 cells treated with chemotherapy drug 5-Fluorouracil (5-FU). As shown in Fig. 4B, both in control or







	UHRF1 IHC	
	Positive	Negitive
ccRCC	23/31	8/31
Adajacent non-tumor	13/31	18/31

*P(ccRCC,non)<0.05 Chi-square(χ2) test

Fig. 1. UHRF1 was overexpressed in ccRCC (A) The mRNA level of UHRF1 was analyzed in 30 paired ccRCC with their corresponding non-tumor tissues by Quantitative RT-PCR. Log2-transformed fold changes of UHRF1 mRNA with respect to non-cancerous specimens were normalized to GAPDH. Fold change>1.5 was considered as up-regulation. (B) The protein level of UHRF1 was analyzed in additional 23 paired ccRCC and their corresponding non-tumor tissues by Western blot (WB) with anti-UHRF1 antibody. (C) IHC analysis of UHRF1 in 31 pair ccRCC and their corresponding non-cancerous tissues. The IHC signal intensities were scored as positive (score 1–2) and negative (score 0). The *p*-values were determined by a X^2 test.

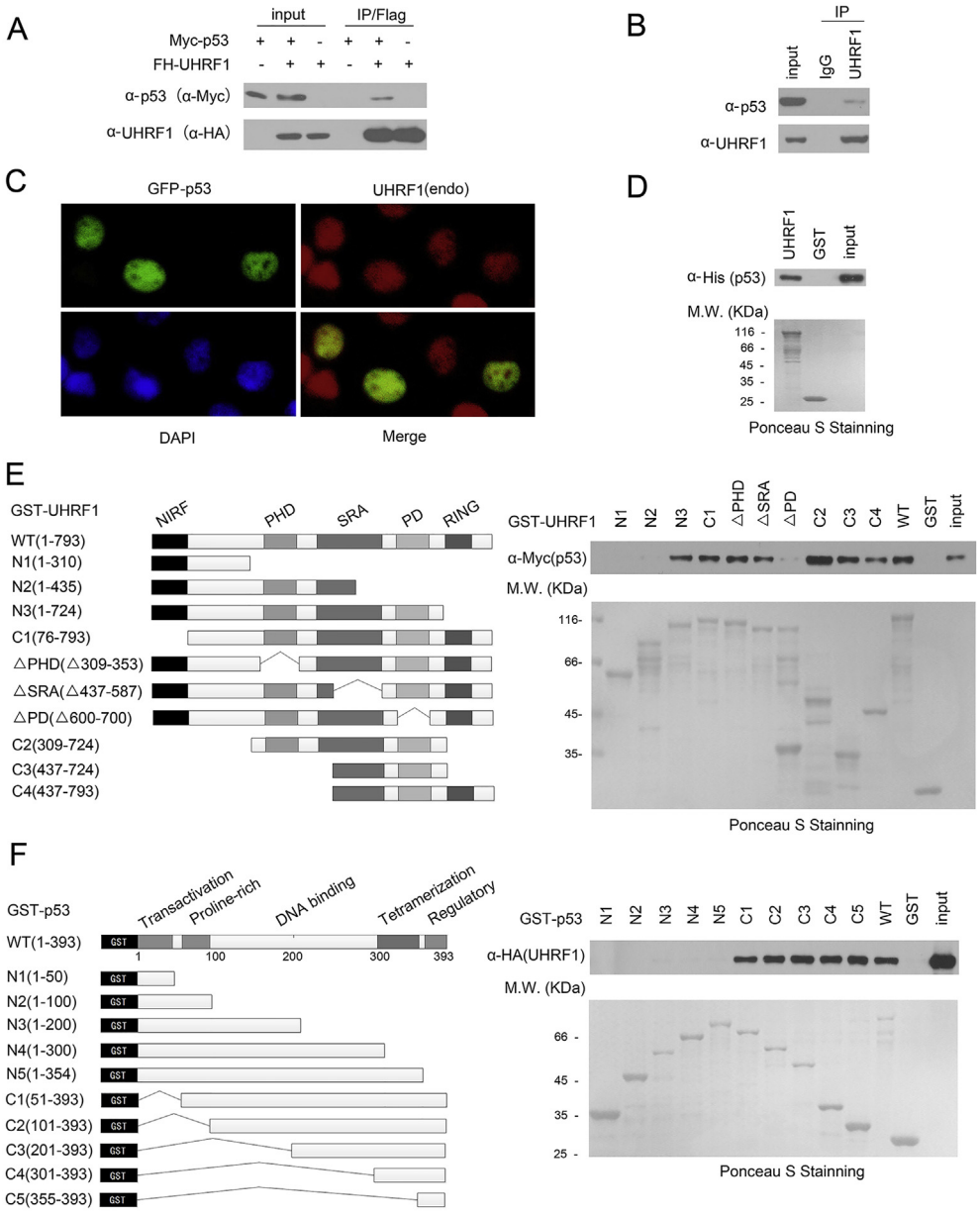


Fig. 2. UHRF1 interacts with p53 both *in vivo* and *in vitro*. (A) 293T cells were co-transfected with Myc-p53 and/or Flag/HA(FH)-UHRF1 constructs, after 24hr, cell lysates were prepared and subjected to co-immunoprecipitation (co-IP) with anti-Flag antibody, and WB analyses with anti-Myc and anti-HA antibodies. (B) Caki-1 cell lysates were prepared for co-IP with anti-UHRF1 antibody and WB analyses with indicated antibodies. (C) Caki-1 cells were transfected with GFP-p53 constructs. After 48 h, the cells were fixed and subjected to immunofluorescence analysis. (D) Bacterially expressed GST fusion UHRF1 protein bound to glutathione-Sepharose beads and incubated with His-tagged fusion p53 protein. Bound His-p53 was detected by WB analyses with anti-His antibody. (E) Schematic model of UHRF1 wild-type and deletion mutants (left panel), and the results of GST pulldown assay (right panel). Bacterially expressed GST fusion proteins of wild-type UHRF1 and the deletion mutants were bound to glutathione-Sepharose beads, respectively, and incubated with lysates of 293T cells transfected with Myc-p53 expression constructs. Bound Myc-p53 was subjected to WB analyses with anti-p53 antibody, and GST-UHRF1 deletion mutant proteins were detected by ponceau S staining. (F) Similar as (E), Schematic model of p53 wild-type and deletion mutants (left panel), and the results of GST pulldown assay (right panel).

UHRF1-depleted cells, p53 protein levels gradually increased in a time-dependent manner without significant difference. However, knockdown of UHRF1 resulted in a marked increase in the accumulation of p53 transcriptional target p21. The 5-FU-induced Lys382 acetylation and Ser15 phosphorylation of p53 were also increased in UHRF1-depleted cells. These results suggest that UHRF1 did not control p53 protein stability, but regulates p53 transactivation activity in DNA damage response. As UHRF1 was found to suppress p53 activation in cells treated with chemotherapy drug 5-FU, we speculated that the potentiation of p53 activation by UHRF1 knockdown might be translated into a phenotypic change in apoptosis. To this end, we transfected Caki-1 cells with either control siRNA or UHRF1 siRNA with/without p53 siRNA followed by 5-FU treatment. Cells were collected 24 h after treatment, stained with PI, and analyzed by flow cytometry for apoptotic cells according to DNA content. As shown in Fig. 4C, basal level sub-G1 content is marginally affected by UHRF1 knockdown. However, 24 h after 5-FU treatment, an average of 28.3% of Caki-1 cells transfected with control siRNA (NC) were apoptotic, whereas about 34.6% (si-UHRF1#1) or 22.9% (si-p53) of cells transfected UHRF1/p53 were apoptotic. Importantly, UHRF1 and p53 co-depletion caused less apoptosis than UHRF1 depleted cells (28.5%

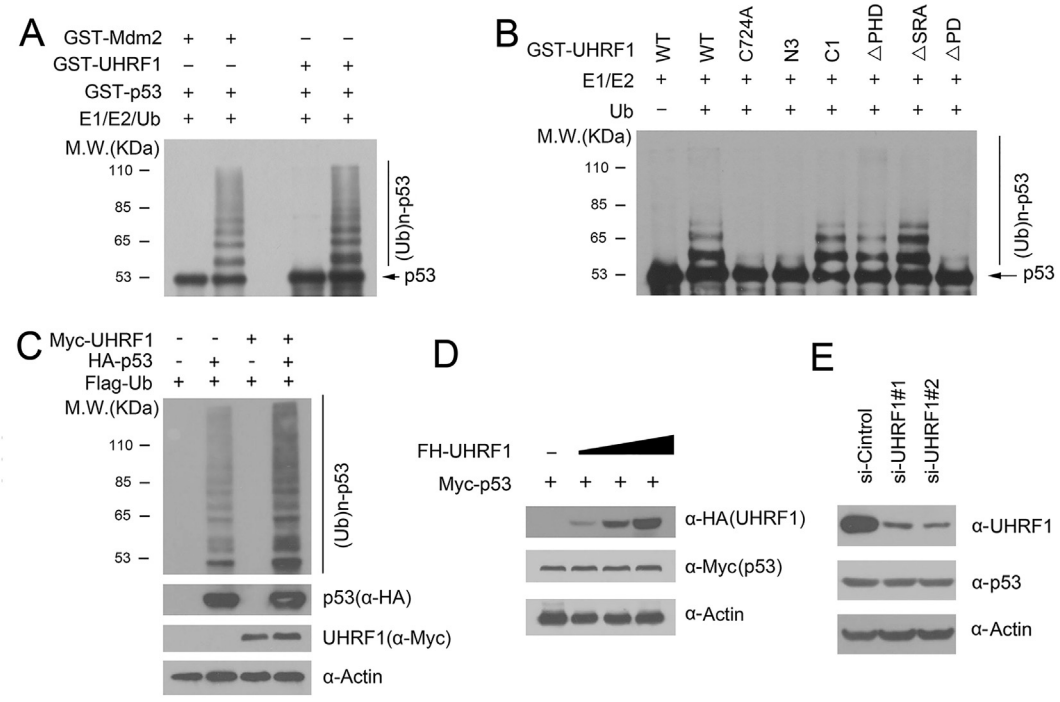


Fig. 3. UHRF1 promotes non-degradative ubiquitination of p53. (A) Bacterially expressed and purified GST-p53 were incubated with GST-UHRF1 or GST-Mdm2 in the presence of E1, E2 (UbcH5a), and ubiquitin. Following the ubiquitination reaction, the poly-ubiquitinated forms of p53 were detected by WB analyses with p53 antibody. (B) UHRF1 wild-type or various mutants were used in the same assay as A. (C) Flag-p53, Myc-UHRF1, and HA-ubiquitin constructs were co-transfected into 293T cells. The poly-ubiquitinated forms of p53 were detected by WB analyses with p53 antibody. (D) 293T cells were transfected with Myc-p53 and increasing amount of FH-UHRF1 constructs. After 24hr, cell lysates were prepared for WB analyses. (E) Caki-1 cells were transfected with control or two independent UHRF1-specific siRNAs, respectively. After 48hr, cells were harvested for WB analyses.

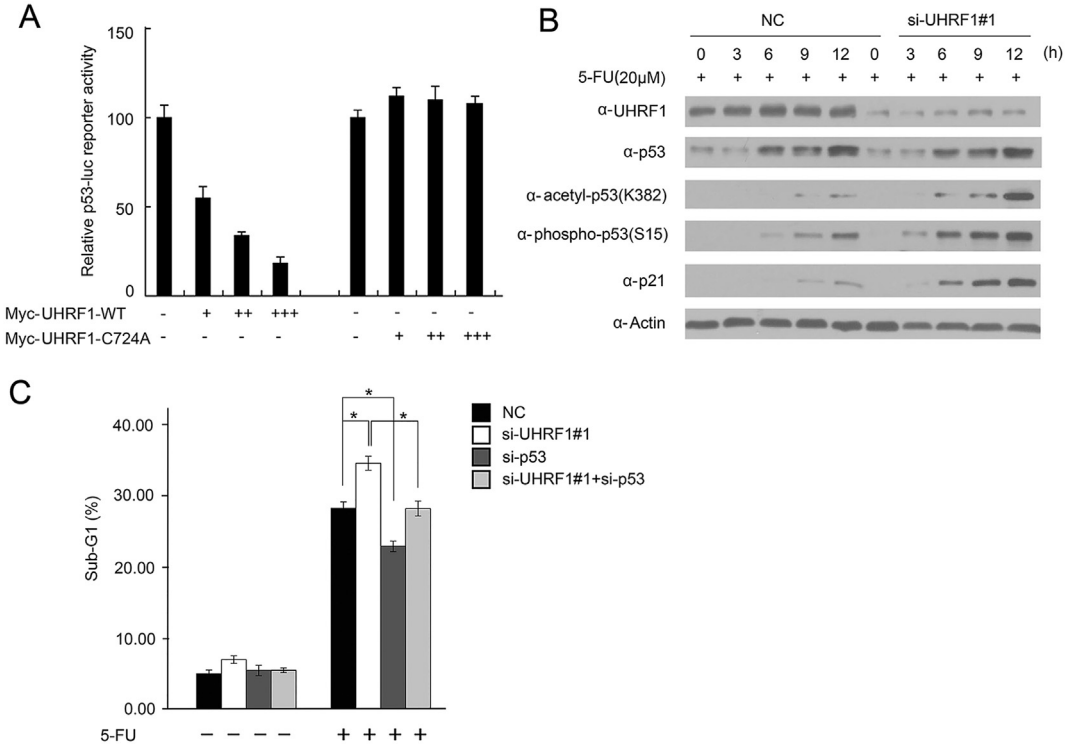


Fig. 4. UHRF1 suppresses p53-dependent transactivation and apoptosis (A) 293T cells were transfected with the p53 responsive reporter (p53-Luc, 100 ng) along with pTK-galactosidase (20 ng) and an increasing amount of both UHRF1-WT and UHRF1-CA mutant with empty construct as indicated. After 24hr, the luciferase activities were measured by luminometer. The mean value (±S.D.) of three independent experiments is shown. (B) Caki-1 cells transiently transfected with siRNAs against UHRF1 or control siRNA (NC) were treated with 5-FU (20μ M) for the indicated time. Cell lysates were detected by WB analyses with indicated antibodies. (C) Caki-1 cells were transfected with siRNAs against UHRF1 (si-UHRF1#1), p53 (si-p53) or control siRNA (NC). After 48hr, the cells were treated with 5-FU (20μ M) for another 24hr, cell apoptosis was measured using PI staining assay. The mean value (±S.D.) of three independent experiments is shown.

Vs 34.6%). Taken together, these results suggest that UHRF1 can suppress chemotherapy drug-induced apoptosis, at least in part, by regulating p53 activation.

4. Discussion

Surgical resection of the primary tumor remains the mainstay of therapy for patients with localized ccRCC disease. However, a high proportion of patients with metastases at diagnosis or relapse following nephrectomy have a poor outcome due to its resistance to chemotherapy and radiotherapy [19]. So, it is very necessary to investigate the molecular mechanism of chemoresistance in ccRCC. Extensive attempts have been made to correlate p53 status with prognosis and chemotherapy response of cancer patients. The presence of p53 mutations was found to predict worse prognosis and reduced response to chemotherapy in many cancers [20]. However, p53 was rarely mutated in ccRCC, suggesting that p53 signaling in this cancer type might be repressed by some other mechanism [21]. In fact, some RCC-derived cell lines maintained wild-type p53 but were not capable of transactivating p53-responsive reporters and endogenous p53-responsive genes [22]. In this study, our findings suggests that a possible pathway may exist through which UHRF1 over-expression promotes p53 inactivation, thus suppresses apoptosis in p53-wildtype RCC cells. Therefore, UHRF1 might be a very promising therapeutic target in ccRCC.

Another intriguing finding from our study was showed that UHRF1 promotes non-degradative ubiquitination of p53. Interestingly, a recent study demonstrated that UHRF1 can promote non-degradative ubiquitination of lysine acetyltransferase Tip60 [23]. Multiple ubiquitin E3 ligases had been reported to reduce p53 stability by targeting p53 for ubiquitin-dependent degradation. However, several studies also suggested non-degradative p53 ubiquitination exists *in vivo* [24]. For example, an atypical ubiquitin E3 ligase, E4F1, was shown to promote K48-linked ubiquitination of p53. E4F1-mediated ubiquitination of p53 does not affect its stability, but instead increases its localization to the chromatin fraction [25]. Another ubiquitin E3 ligase MSL2 specifically ubiquitinates K351 and K357 residues of p53 and targets p53 for nuclear export [26]. Our findings suggest that UHRF1 can be added to the list of E3 ligases that promote non-degradative p53 ubiquitination. Our results showed UHRF1 does not target p53 for nuclear export, since UHRF1 and p53 were co-localize in nucleus (Fig. 1C). Thus, the detailed mechanism and functional impact of UHRF1-mediated p53 ubiquitination remains to be determined.

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