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Mu Zhang
Morsani College of Medicine

Chen Hu
Morsani College of Medicine

Dan Tong
University of Kentucky, dan.tong@uky.edu

Shengyan Xiang
Morsani College of Medicine

Kendra Williams
Morsani College of Medicine

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Ubiquitin-specific Peptidase 10 (USP10) Deubiquitinates and Stabilizes MutS Homolog 2 (MSH2) to Regulate Cellular Sensitivity to DNA Damage*

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Mu Zhang1, Chen Hu1, Dan Tong1, Shengyan Xiang1, Kendra Williams1, Wenlong Bai4,5, Guo-Min Li5,1, Gerold Bepler1, and Xiaohong Zhang1,2

From the 1Department of Pathology and Cell Biology, Morsani College of Medicine, Tampa, Florida 33612, 2Department of Toxicology and Cancer Biology, University of Kentucky College of Medicine, Lexington, Kentucky 40536, 3Cancer Biology and Evolution Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida 33612, and 4Molecular Therapeutics Program, Karmanos Cancer Institute, Detroit, Michigan 48201

MSH2 is a key DNA mismatch repair protein, which plays an important role in genomic stability. In addition to its DNA repair function, MSH2 serves as a sensor for DNA base analogs-provoked DNA replication errors and binds to various DNA damage-induced adducts to trigger cell cycle arrest or apoptosis. Loss or depletion of MSH2 from cells renders resistance to certain DNA-damaging agents. Therefore, the level of MSH2 determines DNA damage response. Previous studies showed that the level of MSH2 protein is modulated by the ubiquitin-proteasome pathway, and histone deacetylase 6 (HDAC6) serves as an E3 ligase of MSH2 to promote its degradation (8). However, the deubiquitinating enzyme (DUB), which counteracts the ubiquitin E3 ligase. However, the deubiquitinating enzymes, USP10 deubiquitinates MSH2 ubiquitin-specific peptidase 10 (USP10) interacts with and stabilizes MSH2. USP10 deubiquitinates MSH2 in vitro and in vivo. Moreover, the protein level of MSH2 is positively correlated with the USP10 protein level in a panel of lung cancer cell lines. Knockdown of USP10 in lung cancer cells exhibits increased cell survival and decreased apoptosis upon the treatment of DNA-methylating agent N-methyl-N’-nitro-N-nitrosoguanidine (MNNG) and antimitabolite 6-thioguanine (6-TG). The above phenotypes can be rescued by ectopic expression of MSH2. In addition, knockdown of MSH2 decreases the cellular mismatch repair activity. Overall, our results suggest a novel USP10-MSH2 pathway regulating DNA damage response and DNA mismatch repair.

Mismatch repair (MMR)3 is a mutation avoidance mechanism that corrects DNA replication errors, and controls homologous recombination (HR) by aborting strand exchange between divergent DNA sequences (1). The MMR activity begins with mismatch recognition, which is carried out either by MutSα (MSH2-MSH6) or by MutSβ (MSH2-MSH3). MutSα recognizes DNA single base pair mismatches and small deletions/insertions, whereas MutSβ recognizes large deletions/insertions (2, 3). MutSo or MutSβ then recruits MutLα (MLH1-PMS2), proliferation cellular nuclear antigen (PCNA), and replication protein A (RPA) to form a complex leading to the recruitment of exonuclease 1 (EXO1) to the strand break. EXO1 then cut the nascent DNA from the nick forward and beyond the mismatch to generate a single strand gap, which is filled by polymerases δ using the parental DNA strand as a template. The repair is accomplished by filling the nick using DNA ligase I. Deletion or mutation of key DNA mismatch repair proteins, such as MSH2 and MLH1, can cause genomic instability (4).

In addition to recognizing DNA mismatches MutSo can recognize certain DNA-damaging agents, such as 6-thioguanine (6-TG)-, N-methyl-N’-nitro-N-nitrosoguanidine (MNNG)-, and cisplatin-induced DNA adducts to trigger apoptosis (5). Thus, the level of MutSo controls cellular sensitivity to DNA damage. It has been reported that the level of MSH2 can be modulated by multiple means. First, when cells are treated with ultraviolet (UV) or phorbol ester (TPA), the mRNA level of MSH2 will be increased (6, 7). Second, MSH2 protein is more stable as a heterodimer with MSH6 than it exists as a monomer (8). Third, protein kinase C (PKC) phosphorylates MutSo and protects it from proteasome-dependent degradation (9). Fourth, histone deacetylase 6 (HDAC6) was recently identified as an E3 ligase of MSH2 to promote its degradation (8). However, the deubiquitinating enzyme (DUB), which counteracts HDAC6 to stabilize MSH2, has remained unknown.

Currently about 100 DUBs have been identified in the human genome and are classified into five families based on their sequence similarity and mechanism of action (10–14). They are 1) the ubiquitin C-terminal hydrolases (UCHs), 2) the ubiquitin-specific proteases/ubiquitin-specific processing proteases (USPs/UBPs), 3) the ovarian tumor proteases (OTUs), 4) the

MNNG, N-methyl-N’-nitro-N-nitrosoguanidine; DUB, deubiquitinating enzyme; USP, ubiquitin-specific peptidase; MSH, MutS homolog.

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1 Present address: Dept. of Biochemistry and Molecular Biology, Norris Comprehensive Cancer Center, University of Southern California Keck School of Medicine, Los Angeles, CA 90033.

2 To whom correspondence should be addressed: Karmanos Cancer Institute, 4100 John R St., Detroit, MI 48201. Tel.: 313-576-8672; Fax: 313-576-8928; E-mail: zhangx@karmanos.org.

3 The abbreviations used are: MMR, mismatch repair; USP10, ubiquitin-specific peptidase 10; DDR, DNA damage response; 6-TG, 6-thioguanine;
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Josephin or Machado-Joseph disease protein domain proteases (MJDs), and 5) the Jab1/MPN domain-associated metalloiso- peptidase (JAMM) domain proteins. The first four families are cysteine peptidases, while the last one comprises of zinc metalloiso-peptidases.

USP10 belongs to the USP family and is identified as a new regulator of p53 in DNA damage response and tumor development (15–17). Yuan et al. found that phosphorylation of USP10 on Thr-42 and Ser-337 by ATM leads to USP10 translocation to the nucleus to stabilize p53 (15). The other USP10-interacting proteins include Ras GTPase-activating protein-binding protein 1 (G3BP1), cystic fibrosis transmembrane conductance regulator (CFTR), and a histone H2A variant, H2A.Z (18–21). Among these USP10 partners, G3BP1 binding to USP10 may block USP10’s enzyme activity. CFTR and H2A.Z are substrates of USP10. USP10 deubiquitinates CFTR to regulate CFTR’s post-endocytic sorting, while USP10 deubiquitinates H2A.Z to transcriptionally activate androgen receptor (AR)-regulated PSA and KLK3 genes (21).

Here, we report MSH2 as a new USP10-interacting protein and a new USP10 substrate. We also reveal a novel USP10-MSH2 pathway regulating MSH2 homeostasis, DNA damage response, and DNA MMR.

Experimental Procedures

Cell Culture and Transfection—All cell lines were grown in Dulbecco’s modified Eagle’s Medium (DMEM) with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 g/ml), except H1299, which was grown in RPMI 1640 medium. Cells were incubated at 37 °C with 5% CO2. The plasmids were grown in DMEM with 10% fetal bovine serum. MSH2 pathway regulating MSH2 homeostasis, DNA damage response, and DNA MMR.

Establishment of USP10-knockdown Stable Clones—For Figs. 2B and 3B, the pGIPZ vector containing shUSP10–2 was transfected into A549 and H1299. One day after transfection, cells were split into 3 dishes. After 24 h, 1 µg/ml puromycin was added into the medium to select positive cells. Ten days later, stable cell lines were subcloned to 60-mm dishes. 0.5 µg/ml puromycin was added to the medium to maintain the stable clones in the subsequent culture.

Establishment of the USP10-knockdown A549 Pool—Addgene’s protocol was followed to produce lentiviral particles and infect A549 cells. Briefly, pGIPZ vector containing shUSP10–1 or shUSP10–2 was co-transfected with psPAX2 and pMD2.G into HEK-293T cells. Lentiviral particles in the HEK-293T cell medium were harvested 24 and 36 h after transfection. Then, the lentiviral particle solution was added into A549 cells. About 24 h after infection, puromycin was added to the medium at the concentration of 1 µg/ml for A549 cells. After a 2-week selection, Western blot analyses were used to determine the knockdown efficiency.

GST Pull-down Assay—GST fusion proteins were purified as previously described (22). For in vitro binding assays, glutathione Sepharose-bound GST-MSH2 proteins were incubated with cell lysates. After washing extensively with PBST (0.1% Tween 20 in PBS), the proteins bound to GST-MSH2 were resolved by SDS-PAGE and immunoblotted with indicated antibodies.

In Vitro Ubiquitination and Deubiquitination Assays—The in vitro ubiquitination assay is described in our previous report (23). Briefly, glutathione-Sepharose-bound GST-MSH2 was incubated with 5 mM ATP, 200 µM HA-UB, and rabbit reticulocyte lysate (RRL) in ubiquitination buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 2.5 mM MgCl2, and 1 mM DTT) for 2 h at 37 °C.
37 °C. Beads were washed with PBST 3 times followed by the deubiquitination buffer (50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA, 10 mM DTT, 5% glycerol) once. Ubiquitinated GST-MSH2 as a substrate was incubated with purified F-USP10 from 293T cells in deubiquitination buffer at 37 °C for 1 h. The reaction was stopped by SDS-loading buffer followed by immunoblotting.

**In Vivo Ubiquitation Assay**—His-MSH2 was co-transfected with empty vector, F-USP10 or F-USP10 (C424A) into 293T cells. Thirty-six hours post-transfection, cells were harvested. Cell pellets were lysed in Buffer A (6 M guanidine-HCl, 0.1 M Na2HPO4/NaH2PO4, 0.01 M Tris-HCl, pH 8.0, 10 mM imidazole, 10 mM β-mercaptoethanol) and 30 μl of Ni-NTA agarose beads were added, and the mixture was rotated at room temperature for 1 h. The beads were sequentially washed with Buffer A, Buffer B (8 μM urea, 0.1 M Na2HPO4/NaH2PO4, 0.01 M Tris-HCl, pH 8.0, 10 mM β-mercaptoethanol), Buffer C (8 M urea, 0.1 M Na2HPO4/NaH2PO4, 0.01 M Tris-HCl, pH 6.3, 10 mM β-mercaptoethanol), and Buffer C plus 0.1% Tween-20, then resolved on SDS-PAGE followed by the anti-UB Western blotting analysis.

**Whole Cell Extract Preparation**—Whole cell extracts were prepared from 6 × 107 cells as described (24). Cells were first washed with buffer A (20 mM Hepes pH 7.5, 5 mM KCl, 0.5 mM MgCl2, 0.1% PMSF, 0.5 mM DTT, 1 μg/ml leupeptin, and 0.2 M sucrose) and lysed in the same buffer without 0.2 M sucrose by passage through a 27-gauge needle. Proteins were precipitated by 65% of ammonium sulfate, collected by centrifugation, resuspended in lysis buffer, followed by dialysis to equilibrium in a buffer containing 20 mM Hepes (pH 7.5), 5 mM KCl, 0.1 mM EDTA, 0.1% PMSF, 0.5 mM DTT, and 1 μg/ml leupeptin.

**Heteroduplex Preparation and MMR Assay**—DNA substrates used in this study are circular heteroduplex DNA containing a unique G·T mismatch and a strand break 3’ to the mismatch (Fig. 6). The substrate was prepared from M13mp18/H11032·mercaptoethanol, and Buffer C plus 0.1% Tween-20, then resolved on SDS-PAGE followed by the anti-UB Western blotting analysis.

**Results**

**USP10 Interacts with MSH2**—We previously identified HDAC6 as an ubiquitin E3 ligase of MSH2 (8). To identify novel MSH2-interacting proteins associated with the ubiquitin-proteasome pathway, we overexpressed HA-MSH2 in 293T cells for 48 h followed by treatment with 50 μM MG132 for 4 h. The HA-MSH2 protein was immunoprecipitated (IP-ed) with anti-HA agarose beads, and the unique bands existing in the anti-HA, but not in the control anti-IgG, immunoprecipitate were excised and analyzed by the liquid chromatography-tandem mass spectrometry (LC-MS/MS). The mass spectrometry analysis identified 14 peptide sequences of USP10, a ubiquitin-specific peptidase (data not shown), suggesting that USP10 exists in the anti-MSH2 immunoprecipitate. Reciprocally, endogenous USP10 was IP-ed by anti-USP10 antibodies in HeLa S3 cells. The immunoprecipitate was resolved by SDS-PAGE, and the bands were excised and examined by LC-MS/MS. MSH2 was identified in the USP10 immunoprecipitate (data not shown), suggesting that USP10 is associated with MSH2.

We next confirmed the interaction between USP10 and MSH2 by performing co-IP assays with anti-USP10 antibodies using 293T and mouse embryonic fibroblasts (MEFs) cell extracts. As shown in Fig. 1A, MSH2 was only detected in the anti-USP10 immunoprecipitates (lanes 3 and 6), but not in the anti-IgG controls (lanes 2 and 5). In a reciprocal fashion, the anti-MSH2 antibody, but not anti-IgG, specifically IP-ed USP10 in HeLa and MEFs (Fig. 1B). Therefore, USP10 and MSH2 indeed interact with each other in vivo.

To determine whether USP10 directly interacts with MSH2 or through other associated proteins, the in vitro GST pull-down assay was carried out. As shown in Fig. 1D, GST-MSH2, but not GST, was able to pull down His-USP10 (lanes 1 and 2). This result strongly indicates a direct interaction between USP10 and MSH2.

We next attempted to map which region of MSH2 binds to USP10. MSH2 can be divided into five domains according to its crystal structure, namely mismatch binding, connector, level, clamp, and ATPase (Fig. 1C) (27). As shown in Fig. 1D, the N terminus of MSH2 (1–378), but not the middle region (200–700) or C terminus of MSH2-(624–934), binds to USP10 directly (lanes 3, 4, and 5). Thus, the major region responsible for the interaction with USP10 is located in the mismatch binding and connector domains of MSH2.

We then examined which region of USP10 binds to MSH2. Human USP10 contains an ubiquitin C-terminal hydrolase domain (412–792) for its deubiquitination activities (Fig. 1E). The N terminus of USP10 has been reported to interact with p53 (15) and G3BP (18). By contrast, as shown in Fig. 1F, the C terminus of USP10 interacts with MSH2 (lane 3).

We also examined whether USP10’s enzymatic activity influences its binding to MSH2. As shown in Fig. 1F, the USP10 catalytically dead mutant (18), USP10 (C424A), interacted with MSH2 to the same extent as the wild type did (lanes 4 and 5), suggesting that the interaction between USP10 and MSH2 is independent of USP10 enzymatic activity.

**USP10 Stabilizes MSH2**—We previously showed that HDAC6 serves as an ubiquitin E3 ligase to promote MSH2’s degradation (8). Here we explored whether USP10 can counteract HDAC6 to stabilize MSH2. As shown in Fig. 2A, in 293T cells, the level of HA-MSH2 increased significantly by overexpressing USP10; in MEFs, the level of endogenous MSH2 increased dramatically by overexpressing USP10, as well. To verify whether USP10 regulates the level of MSH2, we knocked down USP10 by shRNA against USP10 in A549 and H1299 cells. As shown in Fig. 2B, the level of MSH2 was significantly decreased in the USP10 knockdown A549 and H1299 cells
compared with that of MSH2 in the control cells. We then examined whether USP10 influences the stability of MSH2 by measuring the half-life of MSH2. As shown in Fig. 2, C and D, MSH2 half-life in the USP10-knockdown A549 cells is around 6 h, while MSH2 half-life in control cells is more than 12 h, suggesting that USP10 can prolong MSH2’s half-life. We next used a USP10 inhibitor, known as specific and potent autophagy inhibitor-1 (Spautin-1) to treat A549 cells. As shown in Fig. 2E, Spautin-1 was able to decrease the level of MSH2, but not USP10, in a time-dependent manner, suggesting that suppressing the deubiquitinating enzyme activity of USP10 reduces the protein level of MSH2.

We next surveyed a panel of lung cancer cell lines to determine whether there is a positive correlation between USP10 and MSH2 in these cell lines. As shown in Fig. 2, F and G, the expression of MSH2 is positively correlated to the expression of USP10, indicating that USP10 stabilizes MSH2 in lung cancer cells.

**USP10 Deubiquitinates MSH2**—We then determined whether MSH2 is a substrate of USP10. As shown in Fig. 3A,
overexpression of USP10 reduced the ubiquitination of F-MSH2 in A549 and H1299 cells. Conversely, knockdown of USP10 significantly increased the ubiquitination of MSH2 in A549 and H1299 cell lines (Fig. 3B).

To directly examine the deubiquitination activity of USP10 toward MSH2, we utilized a cell-free system. We prepared ubiquitinated GST-MSH2 (Ub-GST-MSH2) as described in “Experimental Procedures” and then performed the deubiquitination assay. As shown in Fig. 3C, F-USP10 efficiently deubiquitinated MSH2 in vitro (compare lanes 2 and 3).

We then tested whether USP10 is able to deubiquitinate MSH2 in vivo. As shown in Fig. 3D, USP10 WT, but not USP10 (C424A), reduced His-MSH2 ubiquitination. To ensure that the ubiquitination signal was indeed from His-MSH2 and not from its associated proteins, His-MSH2 was washed under the denaturing conditions and the ubiquitination status was exam-
Depletion of USP10 Decreases Cellular Sensitivity to MNNG and 6-TG and Decreases DNA Mismatch Repair Activities in A549 Cells—The MSH2-MSH6 heterodimers recognize DNA-damaging agents, such as 6-TG, MNNG, cisplatin, carboplatin, doxorubicin, and etoposide, which form DNA adducts that cannot be removed by MMR (5). It has been well documented that the levels of MSH2 are inversely correlated with 6-TG or MNNG resistance (5). Because USP10 is able to up-regulate the level of MSH2, we set out to test whether USP10 plays a role in regulating MNNG- or 6-TG-mediated cell killing. We depleted the expression of USP10 using two different shRNAs (shUSP10–1 and shUSP10–2) in A549 cells and measured the cytotoxicity of MNNG and 6-TG by MTT assays. As shown in Fig. 4, A and B, knockdown of USP10 using both shUSP10–1 and shUSP10–2 in A549 cells significantly increased cell survival compared with the control cells. To determine whether this increased survival is due to the decrease of apoptosis, we detected the cleavage of poly ADP-ribose polymerase 1 (PARP-1) by Western blotting analyses. As shown in Fig. 1C, knockdown of USP10 with either shUSP10–1 or shUSP10–2 decreased the PARP-1 cleavage upon MNNG and 6-TG treatment compared with the control. To ensure that the increased cell survival is due to the reduction of MSH2, HA-MSH2 was introduced to the A549 pools transduced by shUSP10–1 or shUSP10–2 lentiviruses, and the resulting cells displayed decreased cell survival and increased apoptosis upon 6-TG or MNNG treatment (Fig. 5). Therefore, our data suggest a USP10-MSH2 pathway governing cellular sensitivity to 6-TG and MNNG. In addition, we explored whether depletion of USP10 affects DNA MMR activities. As shown in Fig. 6, knockdown of USP10 in A549 cells significantly reduces the MMR activities, while the addition of the MutSα complex partially...
restores MMR activity. This result indicates that USP10 regulates the cellular MMR activities via modulating the level of MSH2.

Discussion

In this study, we have identified a novel MSH2-interacting protein, USP10, which stabilizes and deubiquitinates MSH2 in vitro and in vivo. The USP10-MSH2 axis regulates the MSH2 and MutSα homeostasis and cellular sensitivity to DNA damage. We previously demonstrated an unexpected E3 ligase activity of HDAC6, which regulates MSH2 proteasome-dependent degradation (8). We have now identified a deubiquitinating enzyme USP10, which counteracts HDAC6’s activity. However, how HDAC6 and USP10 work in concert to regulate MSH2 stability is not clear. Based on our domain mapping data, HDAC6 and USP10 bind to different domains of MSH2. HDAC6 binds to MSH2 C-terminal region (8), while USP10 binds to MSH2 N-terminal region. Therefore, HDAC6 and USP10 may not compete with each other to bind to MSH2.

Unlike one of USP10 substrates, p53, which has a very short half-life, MSH2 has a much longer half-life. We suspect that USP10-mediated deubiquitination of MSH2 may partially account for MSH2 stability. We previously identified four C-terminal lysines (Lys-845, Lys-847, Lys-871, and Lys-892) in MSH2, which can be either acetylated or ubiquitinated (8). We proposed that HDAC6 sequentially deacetylates at these four sites to dissemble the MSH2-MSH6 heterodimers and ubiquitinates the monomer of MSH2. Thus, USP10 may promote deubiquitination of the MSH2 monomer to facilitate MSH2 acetylation and the MSH2-MSH6 dimer formation. We are currently testing this hypothesis in the laboratory.

We also found that USP10 interacts with MSH6 in vivo by the immunoprecipitation assay (data not shown). However, we failed to show that USP10 physically interacts with MSH6. So it is possible that USP10 influences MSH6’s function through a direct interaction with MSH2.

We previously showed that upon 6-TG and MNNG treatment, the level of MSH2 ubiquitination was decreased (data not shown and Ref. 8). Thus, it is likely that USP10 is more activated under the 6-TG or MNNG treatment and deubiquitinates MSH2 more efficiently. Yuan et al., reported that under stress conditions, such as ionizing radiation (IR), USP10 can be phosphorylated by ATM and translocate to the nucleus to stabilize p53 (15). We recently showed that USP10 phosphorylation was increased upon MNNG treatment. A MAPK/CDKs family kinase might be responsible for USP10 phosphorylation (data not shown). Further studies are needed to elucidate how MNNG-induced USP10 phosphorylation affects its enzymatic activity to regulate MSH2 stability.
USP10 Deubiquitmates MSH2

To explore whether USP10 and MSH2 can serve as biomarkers for the sensitivity of chemotherapeutic drugs in lung cancer patients, we examined the mRNA levels of USP10 and MSH2 by qRT-PCR in a cohort of non-small cell lung cancer patients under clinical trials (28). We found that there was a positive correlation between USP10 and MSH2.4 Future studies will determine the protein levels of USP10 and MSH2 in these patients to examine whether USP10 also stabilizes MSH2 in lung cancer patients.

Author Contributions—M. Z. and C. H. performed most of the experiments. D. T. performed the MMR experiment. M. Z. and X. Z. designed the study and wrote the paper. S. X. did the statistical analysis. K. W. revised the manuscript. W. B., G. M. L., and G. B. provided the critical reagents and helped design the experiments. All authors reviewed the results and approved the final version of the manuscript.

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References