UBE2O and USP7 co-regulate RECQL4 ubiquitinylation and homologous recombination-mediated DNA repair

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Abstract
The human RecQ DNA helicase, RECQL4, plays a pivotal role in maintaining genomic stability by regulating the DNA double-strand breaks (DSBs) repair pathway, and is thus, involved in the regulation of aging and cancer onset. However, the regulatory mechanisms of RECQL4, especially its post-translational modifications, have not been fully illustrated. Here, we report that the E2/E3 hybrid ubiquitin-conjugating enzyme, UBE2O, physically interacts with RECQL4, and mediates the multi-monoubiquitinylation of RECQL4, subsequently leading to its proteasomal degradation. Functionally, we showed that UBE2O inhibits homologous recombination (HR)-mediated DSBs repair, and this inhibition depends on its E2 catalytic activity and RECQL4 expression. Mechanistically, we showed that UBE2O attenuates the interaction of RECQL4 and DNA damage repair proteins, the MRE11-RAD50-NBS1 complex and CtIP. Furthermore, we show that deubiquitinylase USP7 interacts with both UBE2O and RECQL4, and in that it antagonizes UBE2O-mediated regulation of RECQL4 stability and function. Collectively, we found a novel regulatory mechanism of ubiquitin-mediated regulation of RECQL4 in HR-mediated DSBs repair process.

Keywords
DNA damage repair, multi-monoubiquitinylation, RECQL4, UBE2O, USP7

Abbreviations: CHX, cycloheximide; CQ, chloroquine; DDR, DNA damage response; DSBs, DNA double-strand breaks; DUBs, deubiquitinylases; HR, homologous recombination; MRN complex, MRE11-RAD50-NBS1 complex; NEM, N-Ethylmaleimide; NHEJ, non-homologous end joining.

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INTRODUCTION

DNA metabolism is an important factor for maintaining genome homeostasis in all organisms. Genomic instability is a hallmark of cancer, premature aging, and neurological disease. The underlying molecular mechanisms of genomic instability could be errors in DNA replication, DNA damage, disease-specific DNA repair defects, or a combination of these. Human DNA helicases are the guardians of genomic stability and cellular homeostasis. They unwind double-stranded DNA and play essential regulatory roles in various aspects of DNA metabolism, such as replication, DNA damage repair, recombination, transcription, and chromosome separation.

RECQL4 is one of the five members of the human RecQ family of DNA helicases. This family also includes RECQL1, WRN, BLM, and RECQL5, which have been shown to play versatile roles in the regulation of diseases related to genomic instability, such as hereditary diseases, aging, and tumors, in humans. Mutations leading to a dysfunction of RECQL4 are closely associated with three genetic diseases: Rothmund-Thomson Syndrome (RTS), RAPADILINO (RAPA), and Baller-Gerold Syndrome. Genetic studies have shown that mice with different exon-specific knockouts of RECQL4 show different survival rates and those adult mice with a RECQL4 deficiency suffer from severe bone marrow failure, leading to hematopoietic dysfunction. Furthermore, cells from patients with RTS or from RECQL4 knockout mice showed changes in chromosome number and structure, suggesting that RECQL4 is essential for the maintenance of chromosome stability. RECQL4 has been confirmed to be involved in multiple DNA metabolism pathways, such as DNA replication and DNA damage repair. Mass spectrometry-based proteomic studies have revealed that RECQL4 interacts with multiple DNA replicators, including CDC45, GINS, SLD5, MCM2-7 helicase, and MCM10, and participates in the initiation of DNA replication. RECQL4 was also shown to be involved in double-strand breaks (DSBs) repair spanning both homologous recombination (HR) and non-homologous end joining (NHEJ). In the S/G2 phase, RECQL4 interacts with DNA DSBs sensor, MRE11-RAD50-NBS1 (MRN) complex, and promotes its interaction with CtIP to mediate 5’ end-to-end resection of the DSBs, facilitating HR-mediated repair.

Ubiquitinylation is an important post-translational modification wherein ubiquitin molecules are covalently attached to the substrate proteins. This process consists of three main steps: activation, conjugation, and ligation, and usually requires three types of enzymes: ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). In addition, ubiquitinylation can be reversed through deubiquitinylation, wherein deubiquitinylases (DUBs) remove the ubiquitin molecules from the substrate proteins. The attachment of a single ubiquitin molecule to one or multiple lysine residue(s) on a substrate protein is called monoubiquitinylation or multi-monoubiquitinylation, respectively. Alternatively, polyubiquitinylation involves the attachment of a series of ubiquitin molecules to substrate proteins and is further divided into homotypic and heterotypic polyubiquitinylation. Polyubiquitin chain is formed by the conjunction of the C-terminal glycine of a distal ubiquitin to the first methionine (M1) or to a lysine residue, including K6, K11, K27, K29, K33, K48, and K63, of a proximal ubiquitin, resulting in eight different types of homotypic polyubiquitin linkages and thousands of potential heterotypic polyubiquitin linkages. We are starting to acknowledge the fact that different types of ubiquitinylations have multiple effects on target proteins. For example, mono- and multi-monoubiquitinylation alter the stability, subcellular location, and protein-protein interactions of the substrate proteins, while attachment of K48-linked polyubiquitin chains mainly causes proteasomal degradation of the substrate proteins. The determination of ubiquitinylation type on a specific protein is decided by the coordinated action of E2 and E3 enzymes. Therefore, studying the E2, and E3 enzymes, and DUBs that act on a specific protein is of great significance for exploring how this protein is regulated by ubiquitin signaling.

Several studies have shown that RECQL4 is highly expressed in multiple cancers, including gastric cancer, breast cancer, and bladder cancer, and promotes DNA end resection, increases HR-mediated DNA repair and cell survival after ionizing radiation, however, little is known about the regulation mechanisms of RECQL4 in cells, especially at the post-translational level. In this study, we utilized a mass spectrometry-based proteomic method to study the interactome of RECQL4 and found that the E2/E3 hybrid ubiquitin-conjugating enzyme UBE2O and ubiquitin specific protease 7 (USP7) interact with RECQL4. Protein interaction mapping analysis identified the Sld2-like domain of RECQL4 required for interacting with UBE2O and USP7. UBE2O mediates multi-monoubiquitinylation of RECQL4, causing its proteasomal degradation, and this process can be antagonized by USP7. We also found that UBE2O inhibits HR-mediated DSBs repair, which depends on its E2 catalytic activity and the expression of RECQL4. Mechanistically, we show that UBE2O inhibits the interaction between RECQL4 and the DNA damage response (DDR) proteins: MRN complex and CtIP, therefore, attenuating the RECQL4-regulated ability of the MRN complex to recruit CtIP for HR-mediated repair. Additionally, we found that USP7 antagonizes the UBE2O-regulated stability and function of
REOQL4. In conclusion, our study provides insight into a novel ubiquitin-mediated regulation of REOQL4 and HR-mediated DSBs repair.

2 MATERIALS AND METHODS

2.1 Plasmids, cell culture and transfection

The full-length human REOQL4, CtIP, TRIM26, and USP7 were amplified from cDNAs of HEK293T and subsequently cloned into CMV promoter-based Flag or Myc tagged vectors. The full-length and truncated versions of pCR3.1-UBE2O-Myc, pCR3.1-UBE2O-Cys1040-mutated (CS)-Myc and the lentiviral plasmid expressing specific shRNAs for human UBE2O have been described previously. The siRNA kit genOFF h-UBE2O_2500A for knockdown was cloned into a home-made vector. Three REOQL4 shRNAs sequences from Sigma (MISSION® shRNA) were tested, and the effective siRNA (shRECQL4: GGCTCAAGGCCAATCTGAAAG) was used to construct an inducible shRNA plasmid as described before. Negative control siRNA (siNC: CCGGACCUGUUAGAAGAAUGUAAA) and siRNA specific to USP7 (siUSP7: CCGGGACCUGUUAGAAGAAUGUAAA) were purchased from Sangon Biotech, China. Lentiviral vectors expressing shRNAs for REOQL4 knockdown were cloned into a homemade vector. Three REOQL4 shRNAs sequences from Sigma (MISSION® shRNA) were tested, and the effective siRNA (shRECQL4: GGCTCAAGGCCAATCTGAAAG) was used to construct an inducible shRNA plasmid as described before. Negative control siRNA (siNC: UUCUCCGAACGGUGACACGU) and siRNA specific to USP7 (siUSP7: CCGGGACCUGUUAGAAGAAUGUAAA) were purchased from Sangon Biotech, China. For the truncated versions of REOQL4, the indicated regions were cloned into N-terminal Flag-tagged pCR3.1 vector. The WT and KR (only lysine mutated to arginine) ubiquitin plasmids were amplified from pRK5-HA-Ubiquitin-WT (A gift from Ted Dawson, addgene plasmid: 17608) and were cloned into N-terminal HA-His-tagged or Myc-His-tagged pCR3.1 vector. The KO and K only ubiquitin plasmids were amplified from pRK5-HA-Ubiquitin-KO (A gift from Ted Dawson, addgene plasmid: 17603) and were cloned into N-terminal HA-His-tagged pCR3.1 vector. pDRGF and pCBASceI were gifts from Maria Jasin (Addgene plasmid: 26475 and 26477). All plasmids were confirmed by DNA sequencing. Primers used for plasmids construction are showed in Table S1.

The human embryonic kidney cell lines HEK293T and the human osteosarcoma cell lines U-2 OS were obtained from ATCC. Gastric cancer cell lines AGS, NCI-N87, HGC-27, and SNU-1 were purchased from the cell bank of the Chinese Academy of Science. HEK293T, U-2 OS, AGS, NCI-N87, and HGC-27 were routinely maintained in Dulbecco’s Modified Eagle’s Medium (DMEM, Hyclone, SH30022.01), and SUN-1 cells were cultured in RPMI medium 1640 basic (Gibco, C22400500BT). Mediums were supplemented with 10% Fetal bovine serum (FBS, HyClone) and 100 U/ml penicillin, 100 μg/ml streptomycin (HyClone, SV30010). All cells were maintained as monolayers in a humidified atmosphere containing at 37°C with 5% CO2 and were routinely tested for mycoplasma (Lonza, LT07-318).

Poly-ethylenimine (PEI, Polysciences) reagent was used for plasmids transfection, and Lipofectamine™ RNAiMAX Transfection Reagent (Thermo Fisher, 13778150) was used for siRNA transfection. Lentiviruses were obtained from the culture supernatant of HEK293T, which were co-transfected with target gene plasmids and three helper plasmids PCMV-VSVG, pMDLg-RRE (gag/pol), and pRSV-REV. Lentiviruses then were used to infect target cells, and stable cell lines were obtained after puromycin selecting as described before. For doxycycline-induced knockdown experiments, cells were treated with 200 ng/ml doxycycline for 48 h.

2.2 In vivo ubiquitinylation assays

In vivo ubiquitinylation assays by anti-Flag immunoprecipitation were conducted as previously described. Briefly, HEK293T cells were transfected with indicated plasmids. After 36 h, cells were washed twice with ice-cold PBS containing 10 mM N-Ethylmaleimide (NEM) and lysed in radioimmunooassay buffer (20 mM NaH2PO4, Na2HPO4, pH 7.4, 150 mM NaCl, 1% Triton X-100, and 0.5% sodium deoxycholate) supplemented with 1% SDS, 1× Complete™ Protease Inhibitor Cocktail (Roche, 4693116001) and 10 mM NEM. Lysates were sonicated, boiled at 95°C for 5 min, diluted to 0.1% SDS by radioimmunoassay buffer, and centrifuged at 14 000 rpm at 4°C for 10 min. The supernatant was incubated with anti-Flag M2 affinity agarose beads (Sigma, M8823) for 1.5 h at 4°C. After 3 washes with radioimmunoassay buffer, proteins were eluted with 2× SDS loading buffer at 42°C for 15 min and separated with SDS-PAGE.

For in vivo ubiquitinylation analysis by nickel pull-down (Ni-NTA), method was used as previously described. In brief, HEK293T cells were washed twice with ice-cold PBS containing 10 mM NEM and lysed in a denaturing buffer (8 M urea, 0.1 M Na2HPO4, 0.1 M NaH2PO4, 10 mM Tris-HCl pH 8.0, 10 mM imidazole) supplemented with 10 mM β-mercaptoethanol. Lysates were centrifuged at 14 000 rpm for 10 min at room temperature and incubated with nickel resins at room temperature for 1.5 h, followed by 3 washes with denaturing buffer and proteins were eluted with 2× SDS loading buffer for 15 min at 42°C before being separated with SDS-PAGE and analyzed by immunoblotting.
2.3 | Immunoprecipitation and proteomics sample preparation

Whole cell extracts were prepared as previously described. In brief, cells were lysed by adding 5 times cell pellet volumes of lysis buffer (0.5% NP40, 150 mM NaCl, 50 mM Tris pH 8.0, 10% Glycerol) supplemented with 1× cOmplete™ Protease Inhibitor Cocktail. Lysates were centrifuged at 14,000 rpm for 10 min at 4°C. The protein concentration of the lysates was measured by the BCA assay kit. Equivalent amounts of proteins were boiled with 1× SDS loading buffer for 5 min at 95°C, and resolved by SDS-PAGE and immunoblotting. Immunoblotting was performed using Mini-PROTEIN™ Tetra Cell system, and image was taken using ChemiDoc Imaging systems from Bio-Rad. All images were generated from individual membrane, except the loading controls were probed using the same membrane. Quantification of the image was performed using ImageJ.

2.4 | Mass spectrometry and data analysis

For ubiquitinylation site identification, tryptic peptides were separated using 140 min of total data collection (100 min of 2% to 22%, 20 min 22% to 28%, and 12 min of 28% to 36% gradient of acetonitrile (Thermo, 51101) for peptide separation, following with two steps washes: 2 min of 36% to 100% and 6 min of 100% acetonitrile) with an Easy-nLC 1000 connected online to a Fusion mass spectrometer (Thermo). For RECQL4 and UBE2O interactors identification, tryptic peptides were separated using 140 min of total data collection (100 min of 2% to 22%, 20 min 22% to 28%, and 12 min of 28% to 36% gradient of acetonitrile (Thermo, 51101) for peptide separation, following with two steps washes: 2 min of 36% to 100% and 6 min of 100% acetonitrile with an Easy-nLC 1200 connected online to a Fusion Lumos mass spectrometer (Thermo). Scans were collected in data-dependent top-speed mode with dynamic exclusion at 90 s. Raw data were analyzed using MaxQuant version 1.6.0.1 search against human FASTA database, with label-free quantification and match between runs functions enabled. For ubiquitinylation site identification, the (GlyGly) remnants search was enabled. The output protein list was analyzed and visualized using DEP package as described before.

2.5 | Western blot and antibodies

Cells were lysed in lysis buffer (0.5% NP40, 150 mM NaCl, 50 mM Tris pH 8.0, 10% Glycerol) supplemented with 1× cOmplete™ Protease Inhibitor Cocktail on ice for 30 min. Lysates were centrifuged at 14,000 rpm for 10 min at 4°C. The supernatants were transferred into a new tube and the protein concentration of the lysates was measured by the BCA assay kit. Equivalent amounts of proteins were boiled with 1× SDS loading buffer for 5 min at 95°C, and resolved by SDS-PAGE and immunoblotting. Immunoblotting was performed using Mini-PROTEIN™ Tetra Cell system, and image was taken using ChemiDoc Imaging systems from Bio-Rad. All images were generated from individual membrane, except the loading controls were probed using the same membrane. Quantification of the image was performed using ImageJ.

Antibodies used in this study included c-Myc (Proteintech Cat# 16286-1-AP, RRID:AB_11182162), HA (Sigma-Aldrich Cat# H6908, RRID:AB_260070), Flag (Sigma-Aldrich Cat# F3165, RRID:AB_259529), USP7 (Bethyl Cat# A303-943A-M, RRID:AB_2781591), RECQL4 (Proteintech Cat# 17008-1-AP, RRID:AB_2238324), UBE2O (Bethyl Cat# A301-873A, RRID:AB_1309799), p95/NBS1 (Cell Signaling Technology Cat# 14956, RRID:AB_2798660), RAD50 (Cell Signaling Technology Cat# 3427, RRID:AB_2176936), MRE11 (Cell Signaling Technology Cat# 4847, RRID:AB_10693469), CtIP (Bethyl Cat# A300-487A, RRID:AB_451022), and GAPDH-HRP (Santa Cruz Biotechnology Cat# sc-47724, RRID:AB_627678).

2.6 | RNA extraction and quantitative real-time PCR

Total RNA was extracted using Trizol Reagent (Invitrogen, 15596-026) according to the manufacturer’s instructions. Equal amounts of total RNA were used for reverse transcription to cDNA using the ReverTra Ace (TOYOBO, TRT-101) according to the manufacturer’s protocol. Real-time PCR was performed with SYBR Green detection system (ChamQ Universal SYBR qPCR Master Mix, Q711-02, Vayzme) using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, USA). Primers used in this paper are showed in Table S1.

2.7 | HR reporter assay

HR repair assay was performed as previously described. In brief, for overexpression experiments, HEK293T cells were transiently co-transfected with pDRGFP and pCBASceI plasmids together with the indicated expression plasmids for 72 h. For siRNA-mediated UBE2O and USP7 knockdown, siRNAs were transfected 24 h before co-transfection of pDRGFP and pCBASceI plasmids for
additional 72 h. Cells were harvested and GFP positive cells were detect and counted by a FACS Canto flow cytometer (Beckman counter, CytoFLEX S), a minimum of 30 000 cells were acquired per sample.

2.8  Statistical analysis

Significant differences were calculated using a paired Student’s t-test: ns indicates no significant; *p < .05; **p < .01; and ***p < .001.

3  RESULTS

3.1  UBE2O interacts with RECQL4

To investigate how RECQL4 is regulated at post-translational level, we decided to study the interactome of RECQL4 using a mass spectrometry-based proteomic method. Flag-tagged RECQL4 was stably overexpressed in U-2 OS cells, a commonly used cell line to study DDR, to identify interacting proteins by immunoprecipitation of Flag beads followed with mass spectrometry detection. As shown in Figure 1A and Table S1, 57 proteins interacting with RECQL4 were identified with high confidence (fold change >2 and p < .05). Of these significant interactors, we identified multiple ubiquitin signaling-related proteins, including E3 ubiquitin ligase TRIM26, E2/E3 hybrid enzyme UBE2O, deubiquitinylase USP7, and USP9X, suggesting a potential role of ubiquitin signaling in the regulation of RECQL4. We decided to investigate the interaction between RECQL4 and UBE2O, since (1) we found that another E3 TRIM26 actually decreased RECQL4 ubiquitinylation (Figure S1); (2) and UBE2O was also identified as RECQL4 interacting protein from a previous study.11 We first validated the association between exogenously expressed UBE2O and RECQL4 using co-immunoprecipitation (Figure 1B). Because no commercial RECQL4 and UBE2O antibodies are suitable for immunoprecipitation, we alternatively showed that endogenous UBE2O was co-precipitated with ectopically expressed RECQL4 and vice versa (Figure 1C,D). To determine which domains of RECQL4 and UBE2O are responsible for their interaction, we constructed truncated versions of UBE2O and RECQL4 according to their predicted domains (Figure 1E,F, top panel).32,33 Co-expression of the truncated RECQL4 and UBE2O followed by co-immunoprecipitation showed that the Sld2-like domain of RECQL4 and the conserved region 2 (CR2) domain of UBE2O are responsible for their interaction (Figure 1E,F, bottom panel). Importantly, recombinantly expressed GST-tagged UBE2O-CR2 domain bound to His-tagged RECQL4-Sld2 like domain (Figure 1G). In summary, these results showed that UBE2O directly interacts with RECQL4.

3.2  UBE2O multi-monoubiquitinylates RECQL4

Previous studies have reported that UBE2O acts as an E2/E3 hybrid ubiquitin enzyme to ubiquitinylate SMAD6 and BAP1, and that the Cys1040 residue within the UBC domain is the E2 active site.27,33 We, therefore, performed a ubiquitinylation assay under denaturation condition using 8 M urea buffer to test whether RECQL4 is a ubiquitinylation substrate of UBE2O. As shown in Figure 2A, UBE2O potentiated the ubiquitinylation of RECQL4, when co-expressed with ubiquitin. Importantly, we showed that the E2 active site was necessary for UBE2O-mediated ubiquitinylation of RECQL4, since the catalytically inactive UBE2O (CS) was unable to ubiquitinylate RECQL4 (Figure 2B). We further validated these results using an alternative method, which denatured proteins in 1% SDS containing buffer, followed by a 10-fold dilution for Flag immunoprecipitation (Figure 2C). Consistently, knockdown of endogenous UBE2O in HEK293T cells greatly reduced RECQL4 ubiquitinylation, further validated that UBE2O targets RECQL4 for ubiquitinylation (Figure 2D).

We noticed multiple UBE2O-mediated ubiquitinylation bands in RECQL4 (Figure 2A–C). We, therefore, speculated that UBE2O targets RECQL4 for either polyubiquitinylation or multi-monoubiquitinylation. To address this, we performed ubiquitinylation assays using multiple ubiquitin mutants that could not form a specific polyubiquitin linkage (K6R, K11R, K27R, K29R, K33R, K48R, and K63R). Surprisingly, we found that all the ubiquitin mutants, when co-expressed with UBE2O, could ubiquitinylate RECQL4 in a pattern similar to that of wild type ubiquitin. We should note that the observation of less ubiquitinylated RECQL4 in some ubiquitin mutants is probably caused by the lower expression level of these mutants (Figure S2A). Considering that all these mutants have ability to attach a single ubiquitin to the protein substrate, we speculated that UBE2O might mediate the multi-monoubiquitinylation of RECQL4. We, therefore, used a knockout (KO) mutant of ubiquitin, which has a tagged N-terminal, and is lysine-free. This ubiquitin mutant loses the ability to form any type of polyubiquitinylation linkage. In agreement with our speculation, multiple ubiquitinylation bands of RECQL4, when co-expressed with UBE2O and the KO ubiquitin still could be observed (Figure 2E). Consistently, ubiquitin mutants that form specific polyubiquitin linkage (K6, K11, K27, K29, K33, K48, and K63) also ubiquitinylated
Identification and validation of UBE2O as a RECQL4-interacting partner. (A) Identification of interacting partners of RECQL4. U-2 OS cells stably expressing Flag-RECQL4 were lysed for immunoprecipitation. Purified protein complexes were tryptic digested, and analyzed by mass spectrometry. Box plot was used to show the enriched proteins. Detailed information about all the identified interactors were shown in Table S1. (B–D) Validation of the interaction between UBE2O and RECQL4. (B) HEK293T cells were co-transfected with Flag-RECQL4 and UBE2O-Myc or vector-Myc for 48 h. Cells were harvested for immunoprecipitation with anti-Flag beads and anti-Myc antibody was used to detect the interaction. (C) HEK293T cells were transfected with Flag-RECQL4 or (D) Flag-UBE2O expression plasmids as indicated followed by immunoprecipitation with anti-Flag beads and immunoblotting analysis with anti-UBE2O or anti-RECQL4 antibody, anti-Flag antibody was used to detect the enrichment of (C) RECQL4 and (D) UBE2O. (E) The Sld2-like domain of RECQL4 interacts with UBE2O. Schematic illustration of the truncations of RECQL4 (top panel). HEK293T cells were co-transfected with UBE2O-Myc and Flag-tagged RECQL4 full length, or its truncations as indicated for 48 h. Cells were harvested for immunoprecipitation with anti-Flag beads and immunoblotting analysis with anti-Myc antibody (bottom panel). (F) The CR2 domain of UBE2O interacts with RECQL4. Schematic illustration of the truncations of UBE2O (top panel, red dot indicates the catalytic active site). HEK293T cells were co-transfected with Myc-RECQL4 and Flag-tagged UBE2O full length or its truncations as indicated for 48 h. Cells were harvested for immunoprecipitation with anti-Flag beads and immunoblotting analysis with an anti-Myc antibody (bottom panel). (G) The Sld2-like domain of RECQL4 directly interacts with the CR2 domain of UBE2O. Bacterial expressed GST-UBE2O-CR2 truncation was coupled to GST resins, followed by incubation with bacterial lysis expressing His-RECQL4-Sld2 truncation. Proteins bound to beads were eluted by boiled with 2× SDS loading buffer, and analyzed by immunoblotting with anti-His antibody. All interaction experiments were repeated at least twice, one representative figure was shown.
FIGURE 2  UBE2O multi-mono ubiquitinylates RECQL4. (A) UBE2O promotes RECQL4 ubiquitinylation. HEK293T cells were co-transfected with Flag-RECQL4 and HA-His-ubiquitin, with or without UBE2O-Myc expression plasmid as indicated for 36 h. Cells were harvested and lysed in 8 M urea buffer, and nickel affinity resins were used to perform the pull down (Ni-NTA). Ubiquitinylated RECQL4 was detected by anti-Flag antibody after Ni-NTA. (B and C) E2 active site is required for UBE2O-mediated ubiquitinylation of RECQL4. HEK293T cells were co-transfected with Flag-RECQL4 and HA-His-ubiquitin, with wild type or Cys1040-mutated (CS) UBE2O-Myc expression plasmids as indicated for 36 h. In vivo ubiquitinylation assay was performed either by (B) Ni-NTA or (C) Flag immunoprecipitation. For ubiquitinylation assay with Flag immunoprecipitation, cells were harvested and sonicated with radioimmunoassay buffer containing 1% SDS, then diluted to 0.1% SDS for immunoprecipitation with anti-Flag beads. (B) Anti-Flag or (C) anti-HA antibody was used to detect ubiquitinated RECQL4. (D) Depletion of UBE2O reduces RECQL4 ubiquitinylation. Inducible UBE2O knockdown HEK293T cells were co-transfected with Flag-RECQL4, with or without Myc-His-ubiquitin plasmids and treated with or without 200 ng/ml doxycycline for 48 h. Cells were harvested for Flag immunoprecipitation ubiquitinylation assay. Anti-Myc antibody were used to detect ubiquitinated RECQL4. (E) UBE2O multi-mono ubiquitinylates RECQL4. HEK293T cells were co-transfected with Flag-RECQL4, UBE2O-Myc and HA-His-ubiquitin wild type or HA-His-ubiquitin-KO plasmids as indicated for 36 h. Cells were harvested for Flag immunoprecipitation ubiquitinylation assay and immunoblotting analyses. Anti-HA antibody was used to detect the ubiquitinylated RECQL4. (F) UBE2O ubiquitinylates RECQL4 at multiple lysine residues. HEK293T cells were co-transfected with HA-His-ubiquitin, and wild type or the indicated mutated K359/361/385/386R (4KR), K332/359/361/385/386R (5KR) or K261/332/359/361/385/386R (6KR) Flag-RECQL4 together with UBE2O-Myc as indicated for 36 h. Cells were harvested for Flag immunoprecipitation ubiquitinylation assay and immunoblotting analyses. Anti-HA antibody was used to detect the ubiquitinylated RECQL4. Intensity of ubiquitinylated RECQL4 was calculated by ImageJ to help the visualization.
UBE2O in a way similar to that of KO ubiquitin and wild type ubiquitin (Figure S2B). These results demonstrated that UBE2O mediates multi-monoubiquitylation of RECQL4.

To identify the potential multi-monoubiquitylated lysine residues in RECQL4, we co-expressed UBE2O, RECQL4, and ubiquitin in HEK293T cells, followed by a two-steps ubiquitylated RECQL4 enrichment and an in-gel digestion-based proteomics identification (Figure S2C). Compared with the sample that co-expressed RECQL4 and ubiquitin, we noted that the intensities of ubiquitylated peptides of RECQL4 at K261, K332, K359, K361, K385, and K386 positions were largely increased in samples that also co-expressed UBE2O, indicating that these lysins may be the ubiquitylation sites (Figure S2D and Table S1). However, ubiquitylation assay used single mutations or combined mutations of identified lysine residues could not completely abolish the UBE2O-mediated ubiquitylation of RECQL4 (Figure S2E,F).

We noted that the most significant inhibition (approximately 50%) of RECQL4 ubiquitylation was observed when 4 lysine residues (359, 361, 385 and 386 lysine residues) were mutated to arginine residues. In addition, no further decrease of RECQL4 ubiquitylation was found when additional lysine residues were mutated (5KR has additional 332 lysine residue mutation, and 6KR has additional 332 and 261 lysine residues mutation) (Figure 2F). In conclusion, we showed that UBE2O multi-monoubiquitylates RECQL4 at multiple sites.

Having found that UBE2O multi-monoubiquitylates RECQL4, we next sought to study the consequences of UBE2O-mediated ubiquitylation of RECQL4. Mono-ubiquitylation is known to alter the functions of substrate proteins in various ways by, for example, affecting their degradation, changing their subcellular localization and trafficking, regulating their activity, or affecting protein-protein interactions. Based on the observations that RECQL4 protein was downregulated when co-expressed with wild type UBE2O and ubiquitin in our ubiquitylation assay (Figure 2B,C), we speculated that UBE2O-mediated multi-monoubiquitylation of RECQL4 leads to its degradation. To explore whether UBE2O governs the stability of RECQL4 through ubiquitylation, we first ruled out the transcriptional regulation of RECQL4 by UBE2O as no significant change in RECQL4 mRNA expression was found in UBE2O over-expressing cells (Figure 3A). In line with the observation in the ubiquitylation assay, compared with overexpression of empty vector or CS mutated UBE2O, ectopically expressed of wild type UBE2O led to a significant down-regulation of RECQL4 protein (Figure 3B). Notably, overexpression of wild type, but not CS mutated UBE2O resulted in an accelerated degradation rate of RECQL4, when U-2 OS cells were treated with cycloheximide (CHX), a protein synthesis inhibitor (Figure 3C). In agreement with overexpression, loss of UBE2O in U-2 OS cells.
resulted in no significant change in RECQL4 mRNA expression but upregulation of endogenous RECQL4 protein (Figure 3D,E). Consistently, the degradation rate of RECQL4 was decelerated in two independent, inducible knockdowns of UBE2O (Figure 3F,G). In additional, we verified this observation in the gastric cancer cell lines AGS, NCI-N87, and HGC-27 (Figure S3A,B). Furthermore, we observed a reverse correlation of UBE2O and RECQL4 protein level in NCI-N87 and SUN-1 cell lines (Figure S3C). These results demonstrated that ubiquitinylation by UBE2O changes the stability of RECQL4.

To determine the pathway that degraded the ubiquitlnylated RECQL4, we treated UBE2O-overexpressing cells with either the proteasome inhibitor MG132 or the lysosomal inhibitor chloroquine (CQ). As shown in Figure 3H, MG132, but not CQ, rescued the UBE2O-dependent RECQL4 degradation, indicating that UBE2O targets RECQL4 for proteasomal degradation. Taken together, our findings strongly support the idea that UBE2O-mediated multi-monoubiquitinylation promotes the proteasomal degradation of RECQL4.

3.4 | USP7 antagonizes UBE2O-mediated ubiquitinylation and degradation of RECQL4

The coordination of ubiquitinylation and deubiquitinylation is pivotal to protein homeostasis. In our RECQL4 interaction screening, we identified two DUBs, USP7 and USP9X, that interact with RECQL4 (Figure 1A). Interestingly, we also found that USP7 interacts with UBE2O in our UBE2O interactome screening experiment (Figure S4 and Table S1). To explore whether USP7 and UBE2O coordinates govern the stability of RECQL4 through ubiquitinylation, we first validated the interaction between endogenous USP7 and ectopic Flag-tagged RECQL4 in U-2 OS cells (Figure 4A). Moreover, co-immunoprecipitation experiment in co-expression of truncated RECQL4 and full-length USP7 cells showed that Sld2-like domain of RECQL4 interacts with USP7 (Figure 4B). Furthermore, we expressed these three proteins individually and used each of them to immuno precipitate the other two proteins in endogenous level. We detected an interaction between USP7, UBE2O, and RECQL4 in HEK293T cells (Figure 4C–E). To test whether the interaction between UBE2O and USP7 is dependent on RECQL4, we performed an interaction assay in inducible knockdown of RECQL4 HEK293T cells and observed that decrease of RECQL4 has no obvious impact on the interactions of UBE2O-USP7 (Figure 4F), indicating that the interaction among UBE2O and USP7 is independent on RECQL4.

Given that USP7 interacts with both UBE2O and RECQL4, we therefore speculated that USP7 might function as a DUB that antagonizes UBE2O-mediated multi-monoubiquitinylation and degradation of RECQL4. To verify our hypothesis, we performed ubiquitinylation assay and found that wild type USP7, but not the catalytically inactive form of USP7 (C223S), inhibited UBE2O-mediated ubiquitinylation of RECQL4 (Figure 4G). Of importance, we showed that UBE2O-mediated decrease of endogenous RECQL4 was rescued by wild type USP7, but not C223S mutated USP7 (Figure 4H). Furthermore, we showed that endogenous RECQL4 protein levels were sustained by the coordinated expressions of UBE2O and USP7 (Figure 4I).

Taken together, our results demonstrated that USP7 interacts with both UBE2O and RECQL4, and antagonizes UBE2O-mediated ubiquitinylation of RECQL4, thus
reversing the regulatory effects of UBE2O on the stability of RECQL4.

3.5 UBE2O inhibits RECQL4-mediated HR repair

Previous study has shown that RECQL4 involves in HR-mediated DSBs repair via promoting CtIP recruitment to the MRN complex at DSBs sites.\(^10\) This prompted us to explore whether UBE2O regulates RECQL4-mediated HR repair. We first investigated whether UBE2O is involved in DNA damage repair. Immunoprecipitation assay showed that ectopically expressed UBE2O interacts not only with RECQL4 and USP7, but also RAD50, NBS1 and γ-H2AX. Of importance, the interaction between UBE2O and γ-H2AX is increased after bleomycin (a single- and double-strand DNA damage inducing agent) treatment in HEK293T cells. This result suggests that the amount of UBE2O that was recruited to the damage site is increased during DNA damage repair and that UBE2O-RECQL4-USP7 complex might co-regulate the DNA damage repair process (Figure S5A,B). Furthermore, overexpression of UBE2O accelerated, while UBE2O knockdown inhibited the degradation of RECQL4 during bleomycin-induced DNA damage process (Figure S5A,B). We also showed that bleomycin-induced DNA damage promotes UBE2O-mediated ubiquitinylation of RECQL4 (Figure S5C,D). Of importance, we observed that dysregulation of UBE2O alters the expression of γ-H2AX during DNA damage repair process (Figure S5A,B). Taken together, these findings provided evidence that UBE2O is indeed involved in the DNA damage repair process.

Next, we examined whether UBE2O has any effect on RECQL4-regulated HR-mediated repair efficiency by an integrated reporter assay. This system uses GFP-positive cells as a readout to evaluate the efficiency of HR-mediated repair after I-SceI endonuclease-induced DSBs (Figure 5B).\(^25\) We verified the positive regulatory effects of RECQL4 on HR-mediated repair,\(^10\) as knockdown of RECQL4 led to a decrease of repair efficiency (Figure S5E, condition f vs. condition b). Interestingly, we found that UBE2O overexpression significantly reduced HR-mediated repair efficiency, and this inhibition depended on the E2 activity of UBE2O (Figure 5C). Consistently, we showed that knockdown of UBE2O increased HR-mediated repair efficiency (Figure 5D). To show whether RECQL4 is required for UBE2O inhibition on HR repair, we overexpressed or knockdown RECQL4 in above-mentioned assays. As shown in Figure 5C,D, replenishment of RECQL4 rescued the reduction in HR-mediated repair efficiency caused by the overexpression of UBE2O, while depletion of RECQL4 reversed the increase of HR-mediated repair efficiency caused by the knockdown of UBE2O. Collectively, these results demonstrated that UBE2O inhibits RECQL4-regulated HR-mediated DSBs repair.

As mentioned above, RECQL4 has been reported involved in HR-mediated repair through interacting with MRN complex, thus promoting MRN complex interacts with CtIP to mediate 5’ end-to-end resection of the DSBs. Because the ubiquitinylation of RECQL4 is increased with bleomycin treatment (Figure S5C,D), we therefore focused on studying the effects of UBE2O in RECQL4 regulated MRN-CtIP complex formation in DSBs condition. We speculated that UBE2O might attenuate MRN-CtIP complex formation through promoting the ubiquitinylation and degradation of RECQL4. To address this, we performed co-immunoprecipitation experiment to test whether UBE2O affects the interaction between MRN complex and CtIP. We found that UBE2O expression inhibited the interaction between CtIP and MRN complex, and this inhibition depended on the E2 activity of UBE2O (Figure 5E, lane1–lane3). More importantly, we showed that overexpression of RECQL4 rescued the inhibition effects of UBE2O on RECQL4-MRN-CtIP complex formation (Figure 5E). Collectively, these results indicated that UBE2O inhibits HR-mediated repair via RECQL4.

3.6 USP7 antagonizes the UBE2O-mediated function regulation of RECQL4

Previous study has shown that USP7 physically associates with the MRN complex and DNA damage checkpoint protein 1 to regulate DDR.\(^34\) Here, we sought to investigate the effects of USP7 on UBE2O-mediated RECQL4 functions during DDR process. We used the HR-mediated repair reporter system to study the effects of USP7 on UBE2O-regulated decrease of HR-mediated repair. As shown in Figure 6A, wild type, but not the C223S mutated USP7 significantly rescued UBE2O inhibited HR-mediated repair, while siRNA-mediated USP7 knockdown exacerbated the effects of UBE2O (Figure 6A). Mechanistically, we showed that the UBE2O-mediated decrease in the expression of RECQL4 and HR repair-related proteins was reversed by the ectopic expression of USP7 (Figure 6B). Furthermore, USP7 also rescued the UBE2O-mediated inhibition of MRN-CtIP complex formation (Figure 6C). To further show that USP7 and UBE2O coordinately regulate HR repair in a RECQL4-dependent manner, we examined the effects of USP7 on UBE2O-mediated HR repair in RECQL4 knockdown cells. As expected, we noticed that USP7 only efficiently rescued UBE2O-inhibited HR-mediated repair in wild type RECQL4 cells, but to a lesser extent in RECQL4 knockdown cells (Figure S5E, condition h vs. condition d). Taken together, this observation suggested that the effects of UBE2O and USP7
**FIGURE 5** UBE2O inhibits RECQL4-mediated HR repair. (A) UBE2O interacts with DNA damage repair response proteins. HEK293T cells expressing Flag-UBE2O were treated with DMSO or 10 μg/ml bleomycin for 1 h before being subjected to Flag immunoprecipitation and detected by indicated antibodies. This experiment was repeated twice and one representative figure was shown. (B) Schematic diagram of HR reporter. (C) UBE2O inhibits HR in a E2 catalytic activity dependent manner. HEK293T cells were co-transfected with pDRGFP and pCBASceI either with empty vector, wild type or CS mutated UBE2O-Myc expression plasmids and Myc-RECQL4 for 72 h. DNA repair efficiency correlating with GFP positive was assessed by FACS. The results were shown % of GFP positive cells (top panel). The graphs show mean ± SD, n = 3. ns indicates non-significant; **p < .01; ***p < .001 (middle panel). Expression levels of UBE2O-Myc and Myc-RECQL4 were detected by immunoblotting (bottom panel). Black arrows indicate myc antibody detected UBE2O and RECQL4. RECQL4 antibody was used to validation of the overexpression of RECQL4. (D) RECQL4 is required for UBE2O depletion increased HR. Inducible RECQL4 knockdown HEK293T cell lines were transfected with control (siNC) or UBE2O siRNA and treated with or without 200 ng/ml doxycycline for 24 h before co-transfection of pDRGFP and pCBASceI for additional 72 h. DNA repair efficiency (top and middle panel) was calculated as Figure 5C and expression levels of UBE2O and RECQL4 were detected by immunoblotting (bottom panel). (E) UBE2O attenuates RECQL4-mediated MRN-CtIP complex formation. HEK293T cells were co-transfected with Flag-CtIP and HA-His-ubiquitin, with or without wild type or CS mutated UBE2O for 36 h, and then treated with 10 μg/ml bleomycin for 1 h. Cells were harvested for immunoprecipitation with anti-Flag beads and immunoblotting analysis with indicated antibodies. This experiment was repeated twice and one representative figure was shown.
on HR-mediated repair is mediated by the expression of RECQL4.

In summary, we showed that USP7 antagonizes UBE2O-mediated decreased HR efficiency. HEK293T cells were co-transfected with pDRGFP, pCBAScel and UBE2O-Myc together either with empty vector, wild type or Cys223S mutated Myc-USP7 expression plasmids for 72 h. For siRNA-mediated USP7 knockdown, cells were transfected with siUSP7 for 24 h before plasmids were co-transfected. DNA repair efficiency correlating with GFP positive was assessed by FACS. DNA repair efficiency was calculated as Figure 5C. The graphs show mean ± SD, n = 3. ns indicates non-significant; *p < .05; **p < .01; ***p < .001 (top and middle panel). Expression levels of UBE2O and USP7 were detected by immunoblotting (bottom panel). (B) UBE2O and USP7 co-regulate the interaction between RECQL4 and DNA damage repair response proteins. HEK293T cells were co-transfected with Flag-RECQL4 and HA-His-ubiquitin, with or without wild type or Cys1040-mutated (CS) UBE2O-Myc, or together with or without wild type or Cys223S mutated Myc-USP7 for 36 h, and then treated with 10 μg/ml bleomycin for 1 h, and cells were harvested for immunoprecipitation with anti-Flag beads and immunoblotting analysis with indicated antibodies. (C) UBE2O and USP7 co-regulate RECQL4-mediated MRN-CtIP complex formation. HEK293T cells were co-transfected with Flag-CtIP and HA-His-ubiquitin, UBE2O-Myc, together with or without wild type or Cys223S mutated Myc-USP7 for 36 h, and then treated with 10 μg/ml bleomycin for 1 h. Cells were harvested for immunoprecipitation with anti-Flag beads and immunoblotting analysis with indicated antibodies. The interaction experiments were repeated at least twice, one representative figure was shown.

4 | DISCUSSION

In eukaryotic cells, a number of factors have been identified that affect the assembly and disassembly of DDR proteins at DNA breaks sites. RECQL4 is one of these factors that is responsible for both the HR- and the NHEJ-mediated repair pathways by regulating the assembly of the MRN-CtIP complex at the DNA damage sites, and by interacting with the Ku70/Ku80 complex, respectively. Therefore, it is not surprising that RECQL4 plays an important role in tumorigenesis and hereditary diseases. However, little is known about the precise mechanism of RECQL4 regulation in tumor cells, especially at post-translational level. Previous studies have shown that RECQL4 is ubiquitylated by the DDB1-CUL4A E3 ubiquitin ligase complex, acetylated by p300 and CBP acetyltransferase, and deacetylated by the deacetylase SIRT1. In this study,
we revealed that the interaction between UBE2O and USP7 is independent of RECQL4. However, our interaction domain mapping experiments have shown that both UBE2O and USP7 interact with the Sld2-like domain of RECQL4. Further structural investigations are required to address how RECQL4 utilizes the Sld2-like domain to interact with both UBE2O and USP7. In addition, we noticed that the expression level of ectopic protein is higher than endogenous protein, which may cause the uncertainty of our conclusions. To rule out this possibility, we have validated our interaction assays with semi-endogenous immunoprecipitation in a reciprocal manner. In addition, all our functional assays, such as ubiquitinylation and DNA repair, are validated by shRNA and/or siRNA-mediated depletion experiments.

UBE2O is an atypical E2-conjugating enzyme that has been shown to have both E2 and E3 activities. Previous studies have shown that UBE2O mediates monoubiquitinylation, multi-monoubiquitinylation, and K48-linked polyubiquitinylation of protein substrates, and alters their localization or stability. UBE2O-mediated multi-monoubiquitinylation of BAP1 triggers the cytoplasmic localization of BAP1. Here, we observed that UBE2O-mediated multi-monoubiquitinylation of RECQL4 leads to its degradation by the proteasome. This is consistent with the observation that UBE2O multi-monoubiquitinylates and triggers the proteasomal degradation of ribosomal proteins during erythropoiesis. Despite the fact that we identified multiple UBE2O-mediated ubiquitinylation sites in RECQL4 using mass spectrometry, we were unable to create lysine residues mutated RECQL4 with resistance toward UBE2O-mediated ubiquitinylation. It seems that K359, K361, K385, and K386 of RECQL4 are the main ubiquitylation sites of UBE2O, because mutation of these 4 lysine residues causes the most significant inhibition on UBE2O mediated RECQL4 ubiquitinylation. A possible explanation for this observation is that other lysine residues might be ubiquitinylated when these 4 lysine residues are mutated. Previously studies have reported an “alternative ubiquitinylation theory”, which means alternative lysine residues will be ubiquitinylated when primary ubiquitinylation site are unavailable. This theory implies that ubiquitinylation is important for the function of RECQL4. Further experiments, such as those using a lysine deficient RECQL4, might assist in assessing this speculation. On the other hand, ubiquitinylation is reversed by deubiquitinylation to maintain the homeostasis of the substrate protein. In fact, our interaction screening identified two DUBs that interact with RECQL4. We show that USP7 is a DUB that counteracts the UBE2O-mediated ubiquitinylation of RECQL4. We speculate that USP9X might be involved in regulating other E3 ligase-mediated ubiquitinylation of RECQL4.

UBE2O and USP7 regulate the ubiquitinylation of various downstream target proteins, impacting multiple cellular functions, such as differentiation, inflammation, and DDR. For example, UBE2O monoubiquitinylates and multi-monoubiquitinylates SMAD6 and BAP1, respectively, to regulate adipocyte differentiation. UBE2O mediates polyubiquitinylation, and the subsequent degradation of AMPKα2, BMAL1, C-Maf, and MLL, affecting tumorigenesis, clock gene regulation, cell apoptosis, and proliferation. Several

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**FIGURE 7** Model diagram showing UBE2O collaborates with USP7 to regulate RECQL4 and HR-mediated repair. The MRN complex recognizes and binds to DSBs sites, and then RECQL4 is recruited to the damage sites. During this process, UBE2O catalyzes the multi-monoubiquitinylation of RECQL4 and promotes its proteasomal degradation, thereby attenuating RECQL4 binding to DNA damage sites, and also attenuating the recruitment of CtIP mediated by RECQL4 to damage sites. However, USP7 reverses the effect of UBE2O. USP7 enhances the stability of RECQL4 by inhibiting ubiquitinylation of RECQL4, which is mediated by UBE2O. In turn, it facilitates the recruitment of RECQL4 to the sites of DNA damage and facilitates the interaction of MRN and CtIP mediated by RECQL4, therefore promoting HR-mediated repair. X represents unidentified protein that may recruit UBE2O to DSBs sites.
studies have demonstrated that USP7 forms a complex with an E3 ubiquitin ligase to jointly regulate target-mediated functions. For example, USP7 forms complexes with the E3 ubiquitin ligases UHRF1, and TRIM27, the PRC1 component RING1B (RNF2), MDM2, RAD18, and PCNA as well as the viral E3 ubiquitin ligase ICP0, subsequently regulating the maintenance of DNA methylation, apoptosis, PRC1-mediated gene repression, DDR, and host antiviral response.47 In this study, we show that UBE2O and USP7 form a complex and regulate the ubiquitylation and stability of RECQL4. Because of the important roles of RECQL4 in HR-mediated repair. It is, therefore, unsurprising to find that UBE2O and USP7 coordinately regulate HR-mediated repair. In this study, we showed that UBE2O inhibits HR-mediated repair, and this inhibitory effect is dependent on RECQL4 expression (Figure 5C,D). Furthermore, we showed that USP7 rescues the inhibitory effects of UBE2O on HR-mediated repair in a RECQL4 expression dependent manner (Figure S5E). These results together proved that RECQL4 is the intermediate that links USP7 and UBE2O to HR-mediated DSBs repair. However, we should note that USP7 could participate in DNA damage repair process via other intermediators.34 Indeed, we found that UBE2O still inhibits HR-mediated repair in RECQL4 knockdown cells, and overexpression of USP7 partially rescues UBE2O-inhibited HR-mediated repair in RECQL4 knockdown cells (Figure S5E). This result suggests that UBE2O and USP7 may also participate in HR-mediated repair via other proteins.

One interesting question is how UBE2O is translocated to DSBs sites during DNA damage. In our experiment, we observed the interaction between UBE2O and DDR proteins (Figure 5A). However, bleomycin treatment has no obvious effect on the interaction between UBE2O and MRN complex proteins, compared to non-treated cells, suggesting that MRN complex is not responsible for UBE2O to localize to DSBs sites. In addition, we showed that UBE2O disrupts the interaction between RECQL4 and MRN complex. Therefore, we speculate that UBE2O might be recruited after RECQL4 have been translocated to the DSBs sites. Another possibility is other repair-related proteins or protein complexes are existed for UBE2O translocated to DSBs sites. Further work is needed to establish the orchestration of the recruitment of UBE2O and other repair proteins to DNA damage foci.

The sensitivity of cancer cells to chemotherapeutic drugs affects the drugs’ therapeutic efficacy, and this is closely related to their ability to repair drug-induced DNA damage. Several reports have confirmed that cells with a deficiency or a mutation of RECQL4 are more sensitive to γ-irradiation/cisplatin than their normal counterparts.48–50 Therefore, UBE2O mediated RECQL4 degradation may sensitize cancer cells to chemotherapy or radiotherapy. However, this is contradictory with a recent study that shows a deficiency of UBE2O enhances Mxi1-mediated radiosensitivity and suppresses tumorigenesis in lung cancer.51 We speculate that UBE2O may exhibit tumor-promoting or inhibition effect in context-dependent manner. Actually, previous studies have showed that UBE2O functions as an oncogene in mouse models of breast and prostate cancers by promoting polyubiquitylation and degradation of AMPKα2.43 Conversely, it is also reported that UBE2O serves as tumor suppressor in multiple myeloma through targeting c-Maf for polyubiquitylation and degradation.45 Therefore, cautions should be taken when UBE2O is served as a target for cancer treatment.

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DISCLOSURES

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

AUTHOR CONTRIBUTIONS

Xiaofei Zhang and Qiuling Huang designed and performed the research; Xiaofei Zhang, Michiel Vermeulen, Dajiang Qin and Duanqing Pei supervised the research; Xiaofei Zhang, Michiel Vermeulen, Dajiang Qin and Duanqing Pei supervised research; Xiaofei Zhang, Michiel Vermeulen, Dajiang Qin and Duanqing Pei supervised research; Xiaofei Zhang and Qiuling Huang analyzed the data; Xiaofei Zhang and Qiuling Huang wrote the paper.

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