Article

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# UCHL3 Regulates the Stability of CDCA5 to Promote DNA Damage Repair and Drive Colorectal Cancer Progression

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**Background: As an important deubiquitinating enzyme, Ubiquitin Carboxyl-terminal Hydrolase L3 (UCHL3) has been considered to play an important role in tumor progression and DNA damage repair. However, its specific function and regulatory mechanism in colorectal cancer remain to be fully elucidated. This study aims to explore the functional role of deubiquitinating enzyme UCHL3 in colorectal cancer, focusing on analyzing its specific mechanism in DNA damage repair and cell cycle regulation by regulating Cell Division Cycle Associated 5 (CDCA5) protein stability, thereby revealing the role of UCHL3 in colorectal cancer and its potential impact on cancer progression.**
**Methods: The experiment consists of normal colon epithelial cells (FHC) and colorectal cancer cell line (SW480) as research subjects to construct UCHL3 knockdown and overexpression models, respectively. The expression levels of UCHL3 and CDCA5 were evaluated using quantitative real-time PCR (qRT-PCR) and Western blot analyses, respectively. Cell viability was assessed using the CCK-8 assay, apoptosis rate and cell cycle distribution were analyzed using flow cytometry, DNA damage level was assessed via comet assay, and Co-IP was used to verify the relationship between UCHL3 and CDCA5, protein interaction of CDCA5, and its ubiquitination level of CDCA5.**
**Results: UCHL3 and CDCA5 were significantly upregulated in CRC cells compared with normal controls ($p < 0.05$). Silencing UCHL3 markedly reduced cell viability ($p < 0.05$), increased apoptosis ($p < 0.05$), enhanced DNA damage ($p < 0.05$), and induced G2/M cell cycle arrest ($p < 0.05$). Conversely, UCHL3 overexpression significantly promoted cell viability ($p < 0.05$), decreased apoptosis ($p < 0.05$), and attenuated DNA damage ($p < 0.05$). Mechanistically, UCHL3 stabilized CDCA5 protein by suppressing its ubiquitination ($p < 0.05$), thereby facilitating DNA damage repair and regulating cell cycle progression.**
**Conclusion: UCHL3 promotes DNA damage repair and inhibits apoptosis by stabilizing CDCA5, thereby driving the progression of colorectal cancer. UCHL3 may become a potential target for treating colorectal cancer.**

**Keywords:** UCHL3; CDCA5; DNA damage repair; cell cycle regulation; colorectal cancer

## Introduction

Colorectal cancer (CRC), ranking among the most prevalent malignancies, remains a top contributor to cancer-related morbidity and mortality worldwide and a leading global health burden [1]. According to the GLOBOCAN 2022 estimates released by the International Agency for Research on Cancer (IARC), CRC represents a major global health burden, with approximately 1.93 million new cases and 903,000 deaths annually, corresponding to 9.6% of all cancer diagnoses and 9.3% of total cancer mortality [2]. In developed countries, the incidence rate of CRC has remained a persistent clinical reality; in developing countries, its incidence rate has also increased significantly in recent years, driven by factors such as the adoption of Western dietary patterns and shifting lifestyles [3]. Despite considerable advances in therapeutic modalities, including surgery, chemotherapy, and targeted agents, the clinical outcome for patients with advanced CRC remains poor, as evidenced by a five-year survival rate below 15% [1,4]. Accumulating evidence indicates that colorectal carcinogenesis and progression are fundamentally driven by genome instability, impaired DNA damage repair mechanisms, and dysregulation of cell cycle control [5,6]. Therefore, in-depth studies for understanding these key biological processes and their regulatory molecules are of great significance to explore new therapeutic targets.

DNA damage repair (DDR) is an important mechanism for maintaining cell genome stability and survival, and its abnormalities are considered to be one of the fundamental contributors for the occurrence of various cancers [7,8]. The DDR pathway mainly maintains cellular homeostasis by identifying DNA damage, activating repair proteins, and clearing damage signals. Among them, the role of

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deubiquitinating enzymes has garnered significant research interest in recent years [9,10]. Ubiquitin Carboxyl-terminal Hydrolase L3 (UCHL3), an important member of the deubiquitinating enzyme family, regulates protein stability and activity by removing the ubiquitination tag of target proteins [11,12]. Accumulating evidence has established Ubiquitin Carboxyl-terminal Hydrolase L3 (UCHL3) as a multifaceted regulator in tumor biology, demonstrating critical involvement in cancer cell proliferation, apoptotic regulation, and DNA damage repair pathways across multiple cancer types [13]. Meanwhile, Cell Division Cycle Associated 5 (CDCA5) has been characterized as a key cell cycle regulator that ensures genomic integrity by mediating sister chromatid cohesion [14]. Notably, CDCA5 shows consistent overexpression in CRC and other malignancies, where its expression pattern is strongly associated with enhanced tumor proliferative capacity [15]. However, the interaction between UCHL3 and CDCA5 in the DDR pathway and its molecular mechanism remains unclear, a critical gap need to be addressed in tumor biology.

Although the roles of UCHL3 and CDCA5 in cancer have been partially revealed, whether there is a functional relationship between the two in CRC and how UCHL3 affects DDR by regulating CDCA5 stability remains unclear. Current understanding of the UCHL3/CDCA5 axis remains incomplete, particularly regarding its precise mechanistic role in modulating CRC cell proliferation, apoptosis, and DNA damage repair. This knowledge gap poses a major barrier to a comprehensive understanding of colorectal carcinogenesis and the translational development of therapies targeting UCHL3/CDCA5. Consequently, systematic investigation of this regulatory axis is crucial for advancing both biological insight and therapeutic strategy in CRC.

This study aimed to elucidate how UCHL3 affects DDR and its mechanism of action on CRC progression by regulating the stability of CDCA5. We used the CRC cell line SW480 to generate small interfering RNA (siRNA) interference and gene overexpression models. These were interrogated via quantitative real-time PCR (qRT-PCR), Western blot, flow cytometry, and comet assays to assess molecular and phenotypic changes. Biological functions of the UCHL3/CDCA5 axis and the molecular interaction between UCHL3 and CDCA5 were explored through co-immunoprecipitation (Co-IP) experiments. The research results will provide new molecular targets and a theoretical foundation for treating CRC.

## Materials and Methods

### *Modeling and Grouping*

The experimental models were established using normal colon epithelial cells (FHC, ATCC CRL-1831, Manassas, VA, USA) and colorectal cancer cells (SW480, ATCC CCL-228, Manassas, VA, USA). All cell lines were authenticated by short tandem repeat (STR) profiling and confirmed to be free of mycoplasma contamination. Multiple interference and overexpression experiments were designed to explore how UCHL3 regulates CDCA5 stability and its role in colorectal cancer. SW480 cells were divided into several groups following transfection, with FHC cells serving as normal controls. Specifically, the negative controls included siNC (transfected with control siRNA) and oeNC (transfected with empty pcDNA3.1 vector). The experimental group (si-UCHL3 group) down-regulated its expression by transfecting UCHL3-specific siRNA. The overexpression experimental groups (oe-UCHL3 group and oe-CDCA5 group) up-regulated the expression of UCHL3 or CDCA5 by transfecting pcDNA3.1-UCHL3 or pcDNA3.1-CDCA5 plasmids, respectively. In addition, the combined experimental group (si-UCHL3+oe-CDCA5) was simultaneously transfected with UCHL3-specific siRNA and CDCA5 overexpression plasmid. The complete sequences of all siRNA constructs, overexpression plasmid inserts (UCHL3 and CDCA5), and their corresponding negative controls are provided in **Supplementary File 1**.

Both FHC and SW480 cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) under standard culture conditions (37 °C, 5% $CO_2$). Transient transfection was performed using Lipofectamine 3000 reagent, followed by sample collection at 48 hours post-transfection for subsequent analyses, including gene/protein expression assays (qRT-PCR and Western blot analyses) and functional assessments (flow cytometry and comet assays).

### *qRT-PCR*

Total RNA was isolated from FHC cells and differentially treated SW480 cells (including siNC, si-UCHL3, oeNC, and oe-UCHL3 groups) using TRIzol reagent (Beyotime Biotechnology, Shanghai, China, R0016). RNA quality was verified by spectrophotometric analysis (ThermoFisher Scientific, Waltham, MA, USA, 840-317400) before reverse transcription into cDNA with a commercial kit (ThermoFisher Scientific, Waltham, MA, USA, SO131). Quantitative PCR amplification was conducted on a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) with SYBR Green chemistry (Beyotime Biotechnology, Shanghai, China, D7268S) under standardized thermal cycling parameters: initial denaturation at 95 °C for 10 min; 40 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. The $2^{-\Delta\Delta Ct}$ method was employed for relative quantification, using $\beta$-actin as endogenous control, with triple technical replicates for each sample. The primer sequences used for quantitative PCR are listed in Table 1.

### *Western Blot Analysis*

Cellular proteins from various treatment groups were extracted using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China, L00399), followed by concentra-

**Table 1. The primers used in the present study.**

| | Forward primer (5′-3′) | Reverse primer (5′-3′) |
|---|---|---|
| *UCHL3* | AATTCCTGGAGGAATCTGTGTC | TCATCTATACTTGGTGCCTCAG |
| *β-Actin* | GGGAAATCGTGCGTGACATTAAG | TGTGTTGGCGTACAGGTCTTTG |

tion determination using bicinchoninic acid (BCA) assay. Protein samples (30 µg per lane) were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Burlington, MA, USA, IPVH00010). After blocking with 5% skim milk powder at room temperature for 1 h, UCHL3 antibody (Abcam, Cambridge, UK, ab126703; 1:10,000), CDCA5 antibody (Abcam, Cambridge, UK, ab192237; 1:2000), γH2AX antibody (Abcam, Cambridge, UK, ab81299; 1:2500), BRCA1 antibody (Abcam, Cambridge, UK, ab131360; 1:2000), CHK2 antibody (Abcam, Cambridge, UK, ab207446; 1:2000), P-CHK2 antibody (Abcam, Cambridge, UK, ab59408; 1:2000) were added and incubated overnight at 4 °C. The next day, the corresponding HRP-labeled goat anti-rabbit secondary antibody (Abcam, Cambridge, UK, ab205718; 1:10,000) was added and incubated at room temperature for 1 h. The protein bands were detected using ECL developer (ThermoFisher Scientific, Waltham, MA, USA, 32106) and imaged with a ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA, USA). β-actin (Abcam, Cambridge, UK, ab8227; 1:5000) was used as an internal reference for protein relative expression analysis using Image J software (version 1.53, National Institutes of Health, Bethesda, MD, USA).

### *Cell Counting Kit-8 (CCK-8)*

Cell proliferation was assessed using the Cell Counting Kit-8 (Beyotime Biotechnology, Shanghai, China, C0037). SW480 cells in logarithmic phase were plated in 96-well plates at 2000 cells/well. Following the addition of 10 µL CCK-8 reagent at 0, 24, 48, and 72-hour intervals, cells were incubated for 2 hours at 37 °C protected from light. Absorbance was measured at 450 nm using a microplate reader (Thermo Fisher Scientific), with cell viability calculated relative to blank controls after background subtraction.

### *Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling (TUNEL) Staining*

Apoptosis was evaluated by TUNEL staining (Beyotime Biotechnology, Shanghai, China, C1091). After 48 hours of transfection, SW480 cells were fixed in 4% paraformaldehyde and processed according to the kit protocol. Following a 1-hour incubation at 37 °C in darkness, nuclei were counterstained with DAPI (Beyotime Biotechnology, Shanghai, China, C1005). Stained samples were visualized under a fluorescence microscope (Olympus, Japan), and the apoptosis rate was quantified by determining the percentage of TUNEL-positive cells.

### *Flow Cytometry*

Flow cytometric analysis was performed to determine cell cycle phase distribution. Transfected SW480 cells were collected after 48 hours, fixed with 70% ice-cold ethanol at 4 °C overnight, and then subjected to staining with 50 µg/mL propidium iodide (Beyotime Biotechnology, Shanghai, China, ST511) and 100 µg/mL RNase for 30 minutes at 37 °C in darkness. Cell cycle analysis was conducted using a BD FACSCanto II flow cytometer (BD Biosciences, USA) with a minimum of 10,000 events recorded per sample. Subsequent DNA content quantification was performed with FlowJo software to determine the percentage of cells in G0/G1, S, and G2/M phases, thereby assessing UCHL3 and CDCA5-mediated cell cycle regulation in SW480 cells.

### *Comet Assay*

DNA damage level was detected by the comet assay. 48 hours after transfection, SW480 cells were collected, and a single cell suspension was prepared, mixed with low-melting-point agarose (Beyotime Biotechnology, Shanghai, China, ST466), and spread on comet slides. After solidification, it was placed in lysis buffer at 4 °C overnight. After the slides were treated with alkaline buffer (pH >13, 30 minutes), DNA fragments were separated under electrophoresis conditions, then neutralized with neutral buffer and stained with DAPI. Comet images were captured using a fluorescence microscope (Olympus, Japan). A minimum of 50 comets per sample were analyzed using the ImageJ software (version 8.0; National Institutes of Health, Bethesda, MD, USA) with the OpenComet plugin. Tail DNA % was calculated as (DNA in tail / total DNA) × 100%. The Olive tail moment was calculated as (Tail DNA %) × (Distance between the head and tail centroids). This metric was used as the primary quantifier of DNA damage.

### *Co-Immunoprecipitation (Co-IP)*

Co-IP was employed to assess the protein interaction between UCHL3 and CDCA5 and simultaneously evaluate the ubiquitination status of CDCA5. After quantification using the BCA method, 500–1000 µg of protein samples were collected and incubated with UCHL3 antibody (UK, abcam, ab241490) and Protein A/G magnetic beads (Beyotime Biotechnology, Shanghai, China, P2080S) at 4 °C overnight. After the immune complex was washed with PBS, the interaction between UCHL3 and CDCA5 was detected by Western blot analysis; in the ubiquitination analysis, the ubiquitination level of CDCA5 was detected using an anti-ubiquitin antibody (US, Santa Cruz Biotechnol-

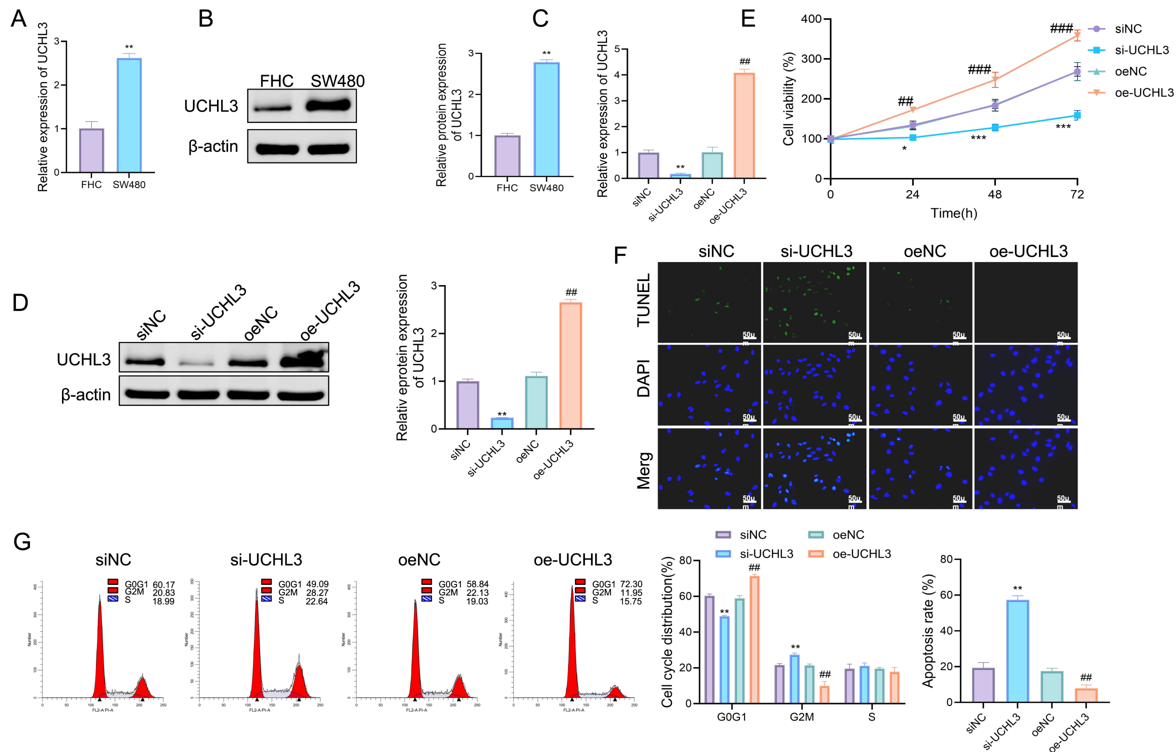


**Fig. 1. Up-regulated expression of UCHL3 in SW480 cells promotes cell viability and inhibits apoptosis and G2/M phase arrest.** (A) Quantitative real-time PCR (qRT-PCR) detects the expression level of UCHL3 in cells of the FHC and SW480 groups. (B) UCHL3 protein expression in FHC and SW480 cells using Western blot analysis. (C) UCHL3 mRNA levels in SW480 transfection groups using qRT-PCR. (D) UCHL3 protein levels in SW480 transfection groups using Western blot analysis. (E) Cell viability of SW480 transfection groups was assessed by CCK-8 assay at 0, 24, 48, and 72 h. (F) Apoptosis rate in SW480 transfection groups was evaluated via TUNEL staining. (G) Cell cycle distribution (G0/G1, S, G2/M phases) in SW480 transfection groups analyzed by flow cytometry. Data are from three independent experiments (n = 3). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs siNC group; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs oeNC group. SW480, colorectal cancer cell line; FHC, normal colon epithelial cells; CCK-8, Cell Counting Kit-8; TUNEL, Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling. UCHL3, Ubiquitin Carboxyl-terminal Hydrolase L3.

ogy, sc-166553; 1:400) to evaluate the regulatory effect of UCHL3 on CDCA5 stability.

### *Cycloheximide (CHX) Chase Assay*

The half-life of the CDCA5 protein was assessed through a CHX (USA, MCE, HY-12320) chase assay. SW480 cells from respective groups were treated with 50 µg/mL CHX, in accordance with the standardized procedures reported in recent studies [16], and followed by whole cell lysis at 0, 2, 4, and 8-hour intervals. CDCA5 protein levels were determined by Western blot analysis (as in Section 1.3), with band intensities quantified using ImageJ software. The CDCA5/$\beta$-actin ratio was calculated to evaluate protein degradation kinetics.

### *Statistical Analysis*

All statistical analyses were performed with GraphPad Prism 8.0.2 (GraphPad Software, San Diego, CA, USA), with results presented as mean ± standard deviation (SD). Data normality and homogeneity of variances were confirmed using Shapiro-Wilk and Levene's tests, respectively. For comparisons between two groups, independent samples *t*-tests were applied; for comparisons involving more than two groups, one-way ANOVA followed by Tukey's post-hoc test was used. Time-dependent measurements were analyzed using repeated-measures ANOVA. Statistical significance was defined as $p < 0.01$, and all experiments included at least three independent replicates to ensure reproducibility.

## Results

### *UCHL3 Is Up-Regulated in SW480 Cells, and Knockdown Induces Apoptosis and G2/M Phase Arrest, and Inhibits Cell Viability*

The qRT-PCR and Western blot results showed (Fig. 1A–D) that compared with normal colon epithelial cell FHC, the mRNA and protein levels of UCHL3 in SW480 cells were significantly increased ($p < 0.05$). Knockdown of UCHL3 (si-UCHL3 group) significantly reduced the expression of UCHL3, while overexpression of UCHL3

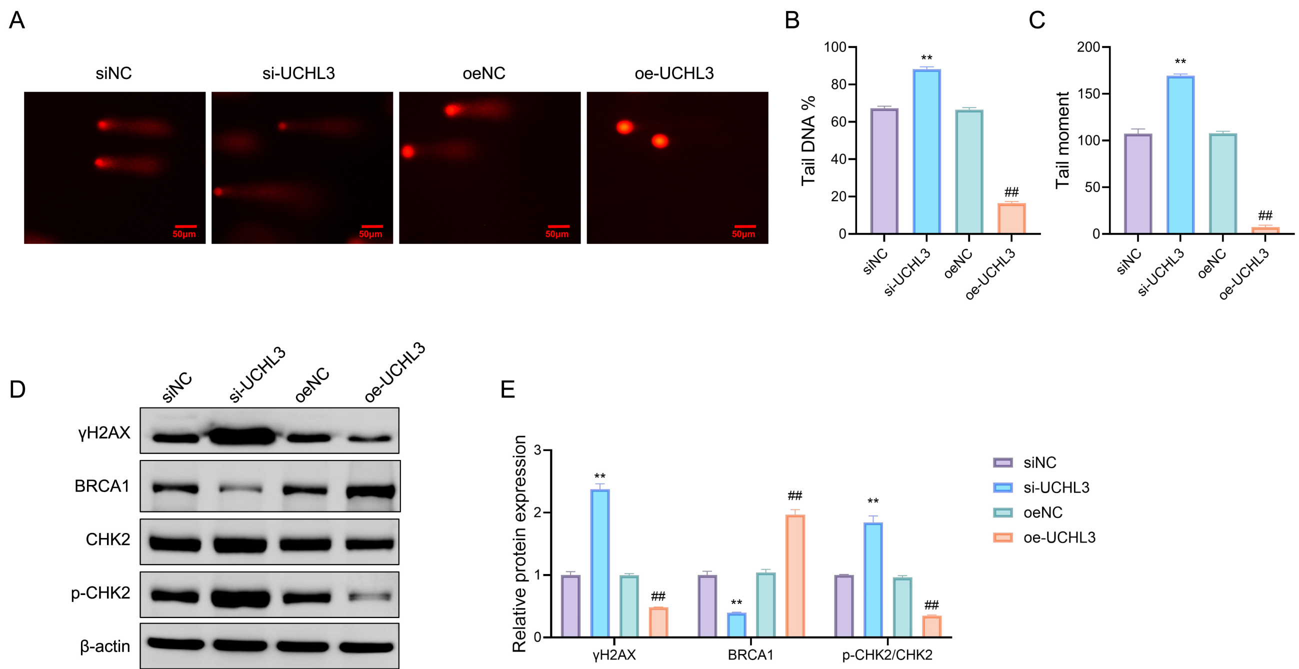


**Fig. 2. Knockdown of UCHL3 promotes DNA damage response in SW480 cells.** (A) Comet assay was used to detect DNA damage levels of cells in the siNC, si-UCHL3, oeNC, and oe-UCHL3 groups. (B) Comet tail DNA levels were analyzed in each group (n = 50 comets). (C) The comet tail distance of each group was analyzed (n = 50 comets). (D) Protein levels of $\gamma$H2AX, BRCA1, CHK2 and p-CHK2 in SW480 cells under different treatments using Western blot analysis. (E) Quantitative analysis of $\gamma$H2AX, BRCA1, and p-CHK2/CHK2. Data are from three independent experiments (n = 3). $^{**}p < 0.01$ vs siNC group; $^{\#\#}p < 0.01$ vs oeNC group.

(oe-UCHL3 group) significantly increased its expression ($p < 0.05$). CCK-8 assay showed (Fig. 1E) that knockdown of UCHL3 inhibited cell viability, while overexpression of UCHL3 significantly enhanced cell viability ($p < 0.05$). TUNEL staining results showed (Fig. 1F) that knockdown of UCHL3 significantly increased apoptosis in SW480 cells, while its overexpression inhibited apoptosis ($p < 0.05$). In addition, flow cytometry analysis showed (Fig. 1G) that knocking down UCHL3 significantly reduced the G0/G1 phase ratio and significantly increased the G2/M phase ratio of SW480 cells, while overexpression of UCHL3 had the opposite effect ($p < 0.05$). These results indicate that UCHL3 plays an important role in CRC by regulating cell viability, apoptosis, and cell cycle.

### *Knockdown of UCHL3 Promotes DNA Damage Response in SW480 Cells*

The results of the comet experiment showed (Fig. 2A–C) that compared with the siNC group, the DNA damage level of cells in the si-UCHL3 group was significantly increased, and both tail moment and tail DNA% were significantly increased; on the contrary, overexpression of UCHL3 was significantly reduced. DNA damage level and the above indicators ($p < 0.05$). Western blot analysis further verified that knocking down UCHL3 enhanced the expression levels of DNA damage-related proteins (Fig. 2D,E), which showed that $\gamma$H2AX and p-CHK2/CHK2 levels were significantly increased in the si-UCHL3 group, whereas the DNA repair-related protein BRCA1 was markedly reduced; overexpression of UCHL3 showed the opposite trend ($p < 0.05$). These results indicate that UCHL3 may play an important role in SW480 cells by regulating DNA damage response-related proteins. Its knockdown can enhance the DNA damage response, while overexpression inhibits this process.

### *Knockdown of UCHL3 Promotes the Degradation of CDCA5 Through Ubiquitination*

Western blot analysis showed (Fig. 3A) that compared with normal colon epithelial cell FHC, the protein expression level of CDCA5 in SW480 cells was significantly increased ($p < 0.05$). Further analysis showed (Fig. 3B) that knockdown of UCHL3 significantly reduced the protein level of CDCA5, while overexpression of UCHL3 significantly increased the protein level of CDCA5 ($p < 0.05$); there was no significant difference between the siNC group and the oeNC group. After CHX treatment (Fig. 3C), CDCA5 protein gradually degraded over time in each group. Knockdown of UCHL3 accelerated the degradation of CDCA5 protein, while overexpression of UCHL3 significantly inhibited the degradation of CDCA5 and maintained a high protein level ($p < 0.05$). The Co-IP assay results showed (Fig. 3D) that there was a direct interaction between UCHL3 and CDCA5 in SW480

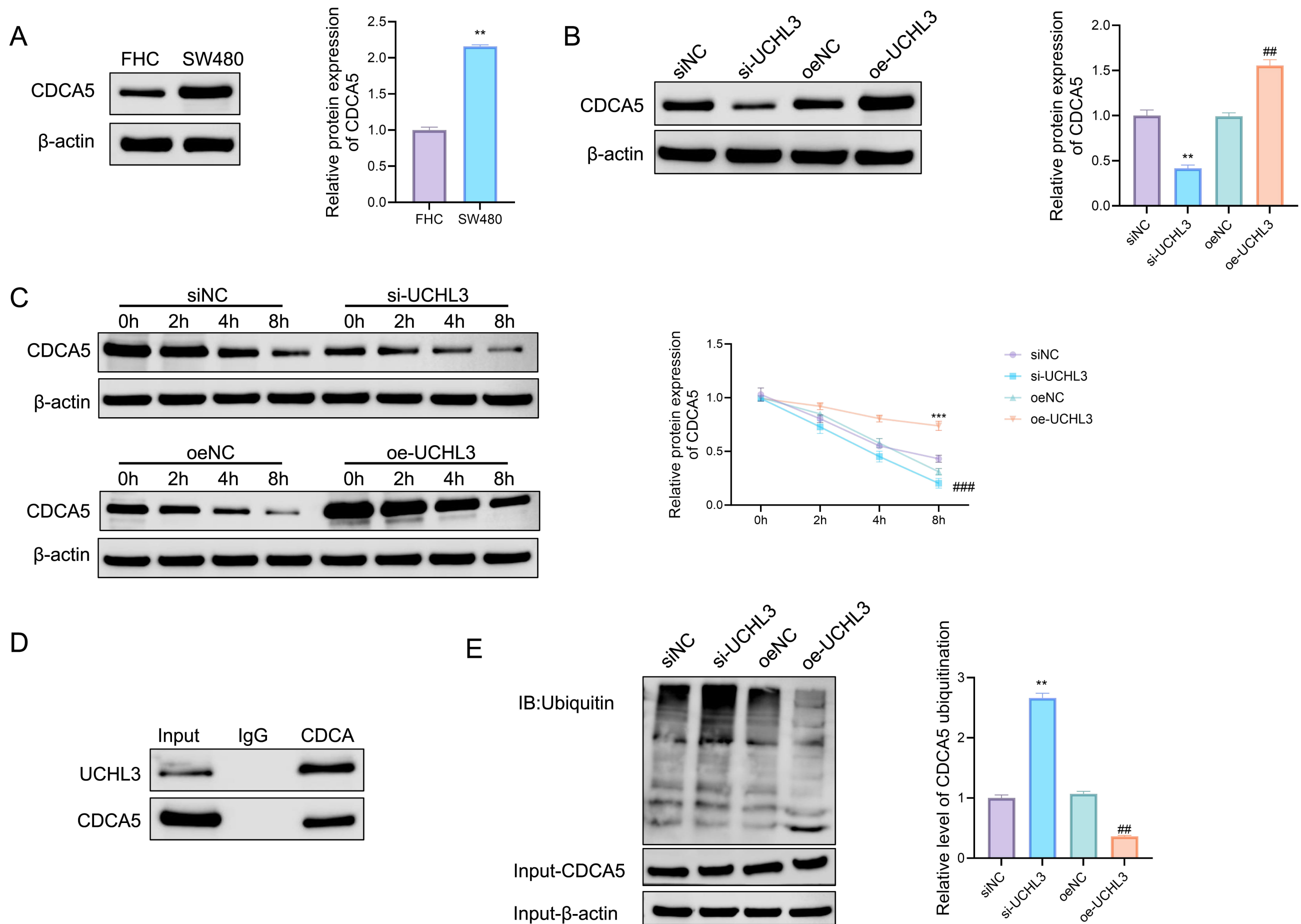


**Fig. 3. UCHL3 affects the protein stability and promotes degradation of CDCA5 by regulating its ubiquitination level.** (A) Western blot analysis was used to detect the protein expression levels of CDCA5 in cells in the FHC group and SW480 group. (B) Western blot analysis was used to detect the protein expression level of CDCA5 in SW480 cells in the siNC, si-UCHL3, oeNC, and oe-UCHL3 groups. (C) CDCA5 protein levels in SW480 transfection groups after CHX treatment (0, 2, 4, 8 h) using Western blot analysis. (D) UCHL3-CDCA5 protein interaction in SW480 cells via Co-IP. (E) CDCA5 ubiquitination levels in SW480 transfection groups via Co-IP. Data are from three independent experiments (n = 3). $^{**}p < 0.01$, $^{***}p < 0.001$ vs siNC group; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.01$ vs oeNC group. CHX, Cycloheximide; CDCA5, Cell Division Cycle Associated 5.

cells (Fig. 3D). Furthermore, Co-IP analysis revealed that UCHL3 knockdown markedly enhanced CDCA5 ubiquitination, whereas UCHL3 overexpression substantially suppressed it (Fig. 3E, $p < 0.05$), with no notable difference observed between the siNC and oeNC groups. These findings demonstrate that UCHL3 modulates CDCA5 stability via deubiquitination, thereby critically influencing SW480 cell function.

### *UCHL3 Promotes CRC Progression by Regulating CDCA5*

Western blot analysis confirmed successful CDCA5 overexpression, showing significantly elevated protein levels in the oe-CDCA5 group compared to oeNC controls (Fig. 4A, $p < 0.05$). Functional assays demonstrated that UCHL3 knockdown substantially impaired cell viability, whereas CDCA5 overexpression enhanced proliferation (Fig. 4B, $p < 0.05$). Notably, concurrent CDCA5 restoration in UCHL3-deficient cells partially rescued the viability defect, with the si-UCHL3+oe-CDCA5 group exhibiting intermediate viability between the si-UCHL3 and oe-CDCA5 groups ($p < 0.05$). Apoptosis analysis revealed parallel trends, as UCHL3 depletion promoted cell death while CDCA5 overexpression exerted protective effects (Fig. 4C, $p < 0.05$). Cell cycle profiling further showed that UCHL3 knockdown induced G2/M arrest, which was ameliorated by CDCA5 co-expression (Fig. 4D, $p < 0.05$). These results indicate that UCHL3 affects cell viability, apoptosis and cell cycle distribution by regulating CDCA5, thereby promoting the progression of CRC.

## Discussion

This study systematically explored the biological function of UCHL3 in CRC and found that UCHL3 is significantly highly expressed in CRC SW480 cells. Its high

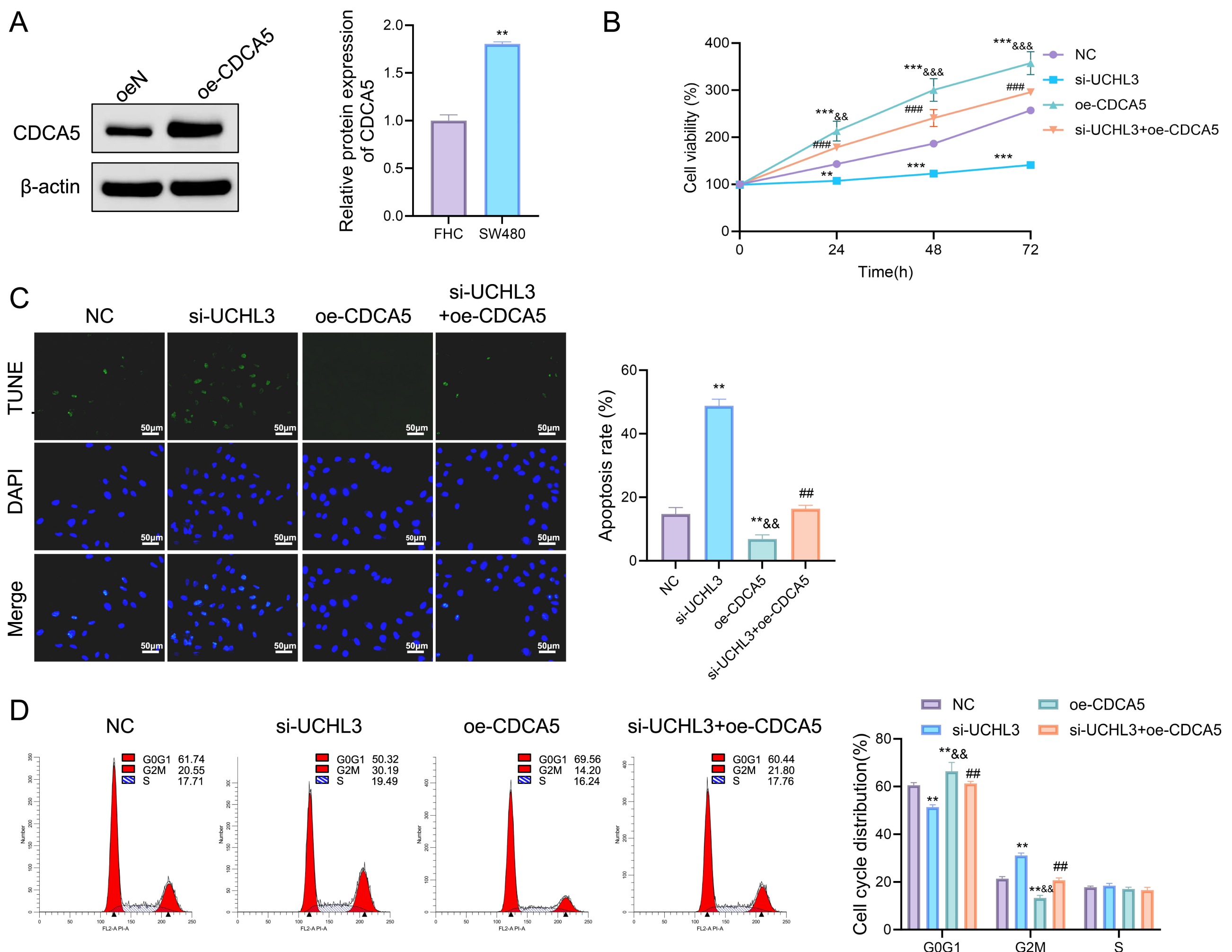


**Fig. 4. UCHL3 promotes the proliferation, inhibits apoptosis and regulates cell cycle distribution of SW480 cells by regulating CDCA5.** (A) Western blot analysis was used to detect the protein levels of CDCA5 in cells in the oeNC group and oe-CDCA5 group. (B) CCK-8 detection (0, 24, 48, 72 h) of SW480 cell viability in the NC, si-UCHL3, oe-CDCA5, and si-UCHL3+oe-CDCA5 groups. (C) TUNEL staining was used to detect the apoptosis level of SW480 cells in the NC, si-UCHL3, oe-CDCA5, and si-UCHL3+oe-CDCA5 groups. (D) Flow cytometry was used to detect the distribution of G0/G1, S, and G2/M cell cycles in SW480 cells in the NC, si-UCHL3, oe-CDCA5, and si-UCHL3+oe-CDCA5 groups. Data are from three independent experiments (n = 3). $^{**}p < 0.01$, $^{***}p < 0.001$ vs NC group; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs si-UCHL3 group; $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ vs si-UCHL3+oe-CDCA5 group.

expression can promote cell viability and inhibit apoptosis and the DNA damage response by regulating the stability of CDCA5, thereby affecting cell cycle distribution. Knocking down UCHL3 significantly reduced the proliferation ability of CRC cells, increased DNA damage levels, and caused cell cycle arrest in the G2/M phase. Further mechanism studies showed that UCHL3 maintains protein stability by inhibiting the ubiquitination of CDCA5, thereby regulating DNA damage repair and cell cycle distribution. These results provide new insights into the role of UCHL3 in CRC and support its possibility as a therapeutic target.

As a pivotal deubiquitinating enzyme, UCHL3's oncogenic roles are increasingly recognized across various cancers, which corroborates our initial finding of its significant upregulation in CRC SW480 cells. For instance, Liu *et al.* [11] demonstrated that UCHL3 promotes bladder cancer progression by stabilizing CTNNB1 and activating the Wnt signaling pathway, highlighting its function as a stabilizer of key oncoproteins through deubiquitination. Similarly, Zhao *et al.* [17] reported that UCHL3 facilitates hepatocellular carcinoma progression by deubiquitinating and stabilizing EEF1A1, further underscoring its role in enhancing tumor malignancy through similar mechanisms. Our findings suggested that UCHL3 stabilizes CDCA5 in CRC aligns with these established patterns, revealing a conserved mechanistic theme while identifying a novel substrate and cancer context.

CDCA5, as a key regulator of sister chromatid cohesion and cell cycle progression, has been implicated in the tumorigenesis of several cancers. He *et al.* [18] con-

ducted a comprehensive pan-cancer analysis, confirming that CDCA5 is significantly overexpressed in multiple solid tumors, including colorectal cancer, and its high expression is strongly associated with poor patient prognosis. This supports our observation of CDCA5 upregulation in CRC cells. At a functional level, Xiong *et al*. [19] elucidated in breast cancer that CDCA5 promotes tumor progression by facilitating the binding of E2F1 to the FOXM1 promoter and activating the Wnt/$\beta$-catenin pathway. Our results are consistent with these findings, as we also identified a crucial role for CDCA5 in driving G2/M phase progression and enhancing DNA damage repair in CRC. However, our study newly positions CDCA5 as a downstream target stabilized by UCHL3, thereby linking UCHL3's deubiquitinating activity directly to CDCA5-mediated cell cycle regulation and genome stability in CRC. This UCHL3/CDCA5 axis represents a previously unreported regulatory mechanism in this malignancy.

Furthermore, our study extends the understanding of UCHL3's role in the DDR. While prior research, such as that by Song *et al*. [13], indicated that UCHL3 inhibition could impair homologous recombination repair in other cancer types, our work mechanistically connects UCHL3 to DDR regulation in CRC via CDCA5. We found that UCHL3 knockdown significantly increased the levels of DNA damage markers ($\gamma$H2AX and p-CHK2) and reduced the expression of the repair protein BRCA1. Given that CDCA5 is known to maintain genome integrity, the degradation of CDCA5 following UCHL3 knockdown likely underlies the observed DDR defects and subsequent G2/M arrest. This establishes a novel functional connection between the UCHL3/CDCA5 axis and the DDR pathway in CRC, suggesting that UCHL3 promotes tumor cell survival not only by fueling proliferation but also by safeguarding genomic integrity through CDCA5.

Although this study revealed the regulatory role of the UCHL3/CDCA5 axis in CRC, there are still some limitations. First, this study was mainly based on *in vitro* cell experiments and lacked verification of animal models and clinical samples, so the specific role of the UCHL3/CDCA5 axis in the *in vivo* environment warrants further investigation. Second, although the study revealed the effects of the UCHL3/CDCA5 axis on DNA damage repair and cell cycle, its downstream signaling pathways and other possible targets have not been fully elucidated. In addition, this study failed to clarify the role of the UCHL3/CDCA5 axis in other types of tumors, limiting further exploration for its wide applicability.

Future studies should verify the role of the UCHL3/CDCA5 axis in the progression of CRC in animal models and clinical samples, and evaluate its potential for clinical application as a therapeutic target. Furthermore, further exploration of the role of UCHL3 in the DDR pathway and its interaction with other DNA damage repair-related factors will support its importance in maintaining tumor genome stability. Developing UCHL3-specific small molecule inhibitors and evaluating their efficacy in the treatment of CRC is also an important direction for future research. The findings of this study not only deepen our understanding of the mechanism of CRC occurrence but also provide a new theoretical foundation for precision therapy targeting the UCHL3/CDCA5 axis.

## Conclusion

This study reveals that UCHL3 maintains protein stability by inhibiting CDCA5 ubiquitination, thereby promoting DNA damage repair, cell cycle progression, and proliferation of CRC cells. The functional mechanism of the UCHL3/CDCA5 axis in CRC provides an important theoretical foundation for the further development of molecular targeted therapy based on the DDR pathway, and UCHL3 is expected to become a potential target for the treatment of CRC.

## Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

## Author Contributions

HLL and LY contributed to conception and design; LY, MJY and HLL were involved in drafting the manuscript and revising it critically for important intellectual content; HLL, LY and MJY made substantial contributions to acquisition of data; and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

## Ethics Approval and Consent to Participate

Not applicable.

## Acknowledgment

Not applicable.

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## Conflict of Interest

The authors declare no conflict of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202638204.6.

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