WD repeat domain 62 (WDR62) promotes resistance of colorectal cancer to oxaliplatin through modulating mitogen-activated protein kinase (MAPK) signaling

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ABSTRACT

WD repeat domain 62 (WDR62) is involved in embryonic brain growth through regulation of glial and neural cell populations. WDR62 is also implicated in the carcinogenesis of various cancers. The role of WDR62 in progression and chemoresistance of colorectal cancer (CRC) was investigated. Firstly, oxaliplatin-resistant CRC cells (HCT116/R and HT29/R) were sequentially exposed to an increasing concentration of oxaliplatin. The results showed that WDR62 was elevated in CRC tissues, and oxaliplatin resistance conferred up-regulation of WDR62 in CRC cells. Knockdown of WDR62 reduced cell proliferation and promoted the apoptosis of oxaliplatin-resistant CRC cells. Moreover, silencing of WDR62 increased fluorescence intensity of γH2AX, and decreased protein expression of p-DNA-PK and Rad51 in the oxaliplatin-resistant CRC cells. The protein expression of p-ERK, p-JNK, and p-p38 in oxaliplatin-resistant CRC cells were down-regulated by knockdown of WDR62. In conclusion, silencing of WDR62 suppressed oxaliplatin resistance and DNA damage repair of CRC cells through inactivation of MAPK signaling.
**Highlights**

- WDR62 was elevated in oxaliplatin-resistant CRC cells.
- Silencing of WDR62 repressed DNA damage repair in oxaliplatin-resistant CRC.
- Silencing of WDR62 suppressed oxaliplatin resistance of CRC cells through inactivation of MAPK signaling.

**Introduction**

Colorectal cancer (CRC) is a common gastrointestinal tumor with high morbidity and mortality [1]. CRC is a heterogeneous disease due to the differences in clinical manifestations, molecular characteristics, and prognosis of the patients [2]. Therefore, CRC is devoid of effective treatment strategies [3]. Surgical resection in combination with adjuvant chemotherapy drugs, such as oxaliplatin, fluorouracil, and folate, is the standard strategy for the prevention of CRC [4]. However, about 50% of CRC patients are resistant to the chemotherapy regimens, which contributes to the recurrence and metastasis of disease [5]. Some patients would develop drug resistance to the chemotherapy, and result in a poor prognosis [5]. Therefore, it is important to elucidate the molecular mechanisms of chemotherapeutic resistance in CRC and identify therapeutic targets to promote chemotherapeutic efficacy.

WD40 duplicate contains 62 (WDR62) was identified as a centrosome associated gene, and was involved in embryonic brain growth through regulation of glial and neural cell populations [6]. WDR62 was associated with the activation of oncogenic signaling in the glial lineage [6]. The oncogenic role of WDR62 was widely reported in distinct tumors [7]. For example, WDR62 was involved in tumorigenesis of bladder cancer [8], and overexpression of WDR62 contributed to centrosome amplification in ovarian cancer [9]. Moreover, WDR62 predicted poor prognosis in lung adenocarcinoma [10]. The exoRBase database showed that exosomal WDR62 was significantly up-regulated in CRC [7]. WDR62 promoted expression of multidrug resistance gene 1 (MDR1) and enhanced the chemoresistance of gastric cancer [11]. However, the role of WDR62 in progression and chemoresistance of CRC remains unknown.

MAPK signaling pathway plays important roles in cell proliferation, survival, death, differentiation, and inflammation [12]. Dysregulation of MAPK signaling was associated with tumor progression and drug resistance [13]. MAPK signaling was abnormally activated in CRC, and inhibition of MAPKs showed clinical benefits in CRC [14]. Since WDR62 has been shown to promote the activation of MAPK signaling and contribute to the drug resistance of gastric cancer [11], WDR62/MAPK axis might also be involved in chemoresistance of CRC. The effect of WDR62 on oxaliplatin resistance of CRC was investigated in this study.

**Materials and methods**

**Bioinformatic analysis**

TIMER (https://cistrome.shinyapps.io/timer/), UALCAN (http://ualcan.path.uab.edu/), and GEPIA (http://gepia.cancer-pku.cn/) were used to detect expression of WDR62 in CRC tissues.

**Cell culture and treatment**

HCT116 and HT29 were cultured in RPMI-1640 medium containing 10% fetal bovine serum (Thermo Fisher, Waltham, MA, USA). To induce oxaliplatin resistance, HCT116 and HT29 were treated with 0.1 μM oxaliplatin (Sigma-Aldrich, St. Louis, MO, USA) for 9 days, and then incubated with 0.5 μM oxaliplatin for 15 days. Followed by cultured in medium containing 1 μM oxaliplatin for 30 days, the cells were exposed to 2 μM oxaliplatin to establish oxaliplatin-resistant CRC cells (HCT116/R and HT29/R) according to previous study [15]. To induce inactivation of MAPK signaling, cells were treated with 20 nM SB 203580 (Thermo Fisher) for 24 hours.

**Measurement of IC50 (half-maximal inhibitory concentration) of oxaliplatin**

HCT116, HT29, HCT116/R, and HT29/R (5X10^3 cells/well) were seeded in 96-well plates, and treated with 1, 2, 4, 8, 16, 32, 64, or 128 μM oxaliplatin...
for 24 hours. Cells were then incubated with CCK8 (Cell Counting Kit-8) (Dojindo, Tokyo, Japan) for another 2 hours, and the absorbance at 450 nm was measured using Microplate Autoreader (Thermo Fisher) according to previous study [15]. Moreover, HCT116/R and HT29/R cells were seeded in 96-well plates, and then transfected with shNC, shWDR62-1# or shWDR62-2# (GenePharma, Suzhou, China) using Lipofectamine 2000 (Gibco, Carlsbad, CA, USA) for 48 hours. Cells were then subjected to CCK8 assay to detect the absorbance at 450 nm.

**Cell proliferation and apoptosis assays**

HCT116/R and HT29/R (5X10^3 cells/well) were seeded in 6-well plates, and then transfected with shNC or shWDR62-2#. Cells were then cultured in the medium with or without 20 μM oxaliplatin for 10 days. Cells were fixed with 4% paraformaldehyde and stained with crystal violet. The number of colony formation was calculated under microscope (Olympus, Tokyo, Japan). To detect cell apoptosis, HCT116/R and HT29/R cells were also transfected with shRNAs and cultured with or without 20 μM oxaliplatin for 48 hours. Cells (1X10^6 cells) were resuspended and incubated with Annexin V-fluorescein isothiocyanate from Annexin V-FITC (Fluorescein)/PI (Propidium Iodide) staining kit (BD Bioscience, San Diego, CA, USA). Followed by incubation with propidium iodide, cells were analyzed under FACS Canto II flow cytometer (BD Bioscience) according to previous study [15].

**Immunofluorescence staining**

HCT116/R and HT29/R cells were transfected with shNC or shWDR62-2# and cultured with or without 20 μM oxaliplatin for 48 hours. Cells (1X10^6 cells) were fixed with 4% paraformaldehyde, and permeabilized with 0.1% Triton X-100. Followed by incubation with 5% bovine serum albumin, cells were treated with anti-γH2AX (Phosphorylated histone H2AX) (1:50; Abcam, Cambridge, MA, USA). FITC-conjugated secondary antibody and DAPI (4′,6-diamidino-2-phenylindole) were then used to incubate the cells before measurement under laser scanning Olympus microscope (Olympus) according to previous study [16].

**RT-qPCR (quantitative reverse transcription PCR)**

RNAs were isolated from cells (1X10^6 cells) using Trizol (Sigma-Aldrich), and then reverse-transcribed into cDNAs. The mRNA expression of WDR62 was detected by SYBR Green Master (Roche, Mannheim, Germany) and analyzed by 2^−ΔΔCq method. Primers used in this study were listed as following: WDR62 (forward: 5′-GGCTTTCTCACCCAATATGAAGC-3′ and reverse: 5′-GCCCTTCACCCAATATGAAGC-3′), GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (forward: 5′-ACCACAGTCCATGCCATCA C-3′ and reverse: 5′-TCCACCACCTGGTTGCTG TA-3′) were used in this study according to previous study [15].

**Western blot**

Cells (1X10^6 cells) were lysed in RIPA buffer (Beyotime, Beijing, China), and the protein samples were segregated using SDS-PAGE. The samples were transferred onto nitrocellulose membranes, and the membranes were blocked in 5% skim milk. Membranes were incubated with primary antibodies: anti-WDR62 and anti-β-actin (1:2000), anti-p-DNA-PK (DNA-dependent protein kinase) and anti-Rad51 (1:2500), anti-p-ERK (extracellular signal-regulated kinase) and anti-ERK (1:3000), anti-p-JNK and anti-JNK (c-Jun N-terminal kinase) (1:3500), anti-p-p38 and anti-p38 (1:4000). The membranes were then incubated with secondary antibodies (1:4500), and subjected to chemiluminescence reagent kit (Beyotime) according to previous study [15]. All the antibodies were purchased from Abcam.

**Statistical analysis**

All the data were expressed as mean ± SEM (Standard Error of the Mean) and analyzed by student’s t test or one-way analysis of variance. p < 0.05 was considered as statistically significant.
Results

Elevation of WDR62 in oxaliplatin-resistant CRC

To investigate the relation between WDR62 and oxaliplatin resistance in CRC, expression of WDR62 was evaluated. Analysis from TIMER showed that WDR62 was significantly up-regulated in CRC tissues compared to normal tissues ($p < 0.001$) (Figure 1(a)). UALCAN (Figure 1(b)) and GEPIA (Figure 1(c)) database also demonstrated higher expression of WDR62 in CRC than the normal tissues. To induce oxaliplatin resistance, HCT116 and HT29 were sequentially exposed to an increasing concentration of oxaliplatin. The IC50 values of oxaliplatin of HCT116 or HT29 cells were 3.401 or 3.095 μM (Figure 1(d)), respectively. However, the IC50 values of oxaliplatin of HCT116/R or HT29/R were 30.84 or 35.495 μM (Figure 1(d)), respectively. HCT116/R and HT29/R expressed higher WDR62 than HCT116 and HT29 (Figure 1(e) and (f)).

WDR62 contributed to oxaliplatin resistance in CRC

To investigate the role of WDR62 in oxaliplatin resistance of CRC, HCT116/R and HT29/R cells were subjected to loss-of-functional assays. HCT116/R and HT29/R cells were transfected with shRNAs to
reduce the expression of WDR62 (Figure 2(a)). shWDR62-2# transfection induced the lower expression of WDR62 and was used in the following assays. Knockdown of WDR62 decreased the IC50 values of oxaliplatin of HCT116/R or HT29/R cells (Figure 2(b)). Moreover, transfection with shWDR62-2# significantly reduced the cell proliferation (p < 0.001) (Figure 2(c)) and enhanced the apoptosis (p < 0.001) (Figure 2(d)) of HCT116/R and HT29/R cells. Treatment with oxaliplatin furtherly inhibited cell proliferation (Figure 2(c)) and promoted the apoptosis (Figure 2(d)) of HCT116/R and HT29/R cells, suggesting that silencing of WDR62 enhanced oxaliplatin sensitivity of CRC through anti-proliferative and pro-apoptotic effects.

**WDR62 contributed to DNA damage repair in oxaliplatin-resistant CRC**

The role of WDR62 in DNA damage repair in oxaliplatin resistance in CRC was then investigated. Transfection with shWDR62-2# up-regulated the

![Figure 2](image-url)
foci of γ-H2AX expression in HCT116/R and HT29/R cells (Figure 3(a)). Moreover, protein expressions of p-DNA-PK and Rad51 in HCT116/R and HT29/R cells were significantly downregulated by knockdown of WDR62 (p < 0.001) (Figure 3(b)), indicating that silencing of WDR62 enhanced oxaliplatin sensitivity of CRC through suppression of DNA damage repair.

**WDR62 contributed to activation of MAPK signaling in oxaliplatin-resistant CRC**

The underlying mechanism involved in WDR62-mediated oxaliplatin resistance in CRC was assessed. Transfection with shWDR62-2# did not affect protein expression of ERK, JNK and p38 in HCT116/R and HT29/R cells (Figure 4). However, the protein expressions of p-ERK, p-JNK, and p-p38 in HCT116/R and HT29/R cells were significantly reduced by transfection with shWDR62-2# (p < 0.001) (Figure 4). HCT116/R and HT29/R cells were transfected with shWDR62-2# in the presence with oxaliplatin and SB 203580 (inhibitor of MAPK). Inhibition of MAPK signaling aggravated WDR62 silencing-induced decrease in cell proliferation (Figure 5(a)) and increase in cell apoptosis (Figure 5(b)) in HCT116/R and HT29/R cells. Moreover, the up-regulation of γ-H2AX foci in HCT116/R and HT29/R cells were increased by treatment with SB 203580 (Figure 5(c)). Inhibition of MAPK also furtherly downregulated protein expression of p-DNA-PK and Rad51 in HCT116/R and HT29/R cells (Figure 5(d)), revealing that silencing of WDR62 inhibited oxaliplatin-resistance of CRC through inactivation of MAPK signaling.

**Discussion**

Pan-cancer analysis revealed the up-regulation of WDR62 in multiple tumors, and WDR62 was associated with infiltration of immune cells and related with poor prognosis [7]. This study for the first time identified the oncogenic role of WDR62 in CRC. In addition, WDR62 was also implicated in the chemoresistance of CRC.

Chemotherapy is widely used in the treatment of CRC, and resistance to oxaliplatin-dependent chemotherapy contributes to the recurrence of CRC [17]. Inhibition of WDR62 has been shown to enhance sensitivity of gastric cancer cells to Adriamycin, cis-diaminodichloroplatinum and
5-fluorouracil [11]. This study found that WDR62 was elevated in CRC tissues compared to the normal tissues, and oxaliplatin-resistant CRC cells showed higher WDR62 expression than CRC cells. The relationship between clinicopathological variables of oxaliplatin-resistant CRC patients and WDR62 expression might provide a potential prognostic or diagnostic biomarker for oxaliplatin-resistant CRC. Knockdown of WDR62 decreased the IC50 values of oxaliplatin of HCT116/R or HT29/R cells, inhibited the cell proliferation and promoted the cell apoptosis, suggesting that silencing of WDR62 enhanced the sensitivity of CRC to oxaliplatin. However, the effects of WDR62 on cell metastasis of oxaliplatin-resistant CRC remains unclear.

Oxaliplatin exerts anti-tumor effect in various cancers through promoting of DNA double-strand breaks, inhibition of DNA synthesis and up-regulation of immunogenic cell death [18]. However, activation of anti-apoptosis signaling pathways and antioxidant glutathione system, inefficiency of drug accumulation, and enhancement of DNA damage repair have been regarded as hypothetical mechanisms involved in drug resistance of CRC [19]. The indicator of DNA double-strand breaks, γ-H2AX, was reduced in oxaliplatin-resistant CRC, whereas the biomarkers of homologous recombination repair, p-DNA-PK and Rad51, were enhanced in the oxaliplatin-resistant CRC [20]. WDR62 regulated H3K9...
trimethylation, and mediated oocyte meiotic maturation [21]. This study identified that WDR62 was associated with DNA damage repair in oxaliplatin-resistant CRC. Silencing of WDR62 increased foci of γ-H2AX expression, while decreased p-DNA-PK and Rad51 proteins in HCT116/R and HT29/R cells, revealing that down-regulation of WDR62 might improve clinical outcomes in oxaliplatin-resistant CRC patients through suppression of DNA damage repair.

Research has shown that oxaliplatin stimulated cell apoptosis of CRC through activation of MAPK signaling, and the inhibition of MAPK attenuated oxaliplatin-induced cell death in CRC [22]. However, hyperactivation of MAPK signaling conferred oxaliplatin resistance to CRC, and MAPK inhibition re-sensitized CRC cells to oxaliplatin [23]. WDR62 functioned as a scaffold protein of JNK, and enhanced the kinase activity of JNK [24]. WDR62 also contributed to multidrug resistance of gastric cancer through activation of MAPK signaling [11]. This study showed that knockdown of WDR62 down-regulated protein expression of p-ERK, p-JNK, and p-p38 in HCT116/R and HT29/R cells, indicating that silencing of WDR62 enhanced the sensitivity of CRC to oxaliplatin through inactivation of MAPK signaling.

**Conclusion**

In summary, WDR62 was involved in chemoresistance and DNA damage repair of CRC. Knockdown of WDR62 inhibited cell proliferation and DNA damage repair and promoted the
apoptosis of oxaliplatin-resistant CRC through inactivation of MAPK signaling. However, the role of WDR62 in oxaliplatin resistance animal model of CRC should be investigated in future studies.

Disclosure statement
No potential conflict of interest was reported by the author(s).

Contribution of authors
Juanjuan Cai designed the experiments and Lingling Su carried them out. Weiwei Luo analyzed and interpreted the data, prepared the manuscript with contributions from all co-authors.

Ethics approval
This article does not contain any studies with human participants or animals performed by any of the authors.

Data availability
The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

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