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Inhibiting the MCM8-9 complex selectively sensitizes cancer cells to cisplatin and olaparib

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MCM8 and MCM9 are paralogues of the MCM2-7 eukaryotic DNA replication helicase proteins and play a crucial role in a homologous recombination-mediated repair process to resolve replication stress by fork stalling. Thus, deficiency of MCM8-9 sensitizes cells to replication stress caused, for example, by platinum compounds that induce interstrand cross-links. It is suggested that cancer cells undergo more replication stress than normal cells due to hyperstimulation of growth. Therefore, it is possible that inhibiting MCM8-9 selectively hypersensitizes cancer cells to platinum compounds and poly(ADP-ribose) polymerase inhibitors, both of which hamper replication fork progression. Here, we inhibited MCM8-9 in transformed and nontransformed cells and examined their sensitivity to cisplatin and olaparib. We found that knockout of MCM9 or knockdown of MCM8 selectively hypersensitized transformed cells to cisplatin and olaparib. In agreement with reported findings, RAS- and human papilloma virus type 16 E7-mediated transformation of human fibroblasts increased replication stress, as indicated by induction of multiple DNA damage responses (including formation of Rad51 foci). Such replication stress induced by oncogenes was further increased by knockdown of MCM8, providing a rationale for cancer-specific hypersensitization to cisplatin and olaparib. Finally, we showed that knocking out MCM9 increased the sensitivity of HCT116 xenograft tumors to cisplatin. Taken together, the data suggest that conceptual MCM8-9 inhibitors will be powerful cancer-specific chemosensitizers for platinum compounds and poly(ADP-ribose) polymerase inhibitors, thereby opening new avenues to the design of novel cancer chemotherapeutic strategies.

KEYWORDS
cancer chemotherapy, cancer-selective hypersensitization, MCM8-9, platinum compound, poly(ADP-ribose) polymerase inhibitor

1 | INTRODUCTION

Platinum-based chemotherapeutic agents such as cisplatin and carboplatin are used widely to treat various cancers, including ovarian cancer, lung cancer, and colon cancer.[1] In particular, patients with ovarian cancers respond well to platinum compounds used as primary chemotherapy. Germline mutations in *BRCA1* or *BRCA2* tumor suppressor genes cause familial breast/ovarian cancer[2–6]; however,

BRCA1/2-deficient cancer cells are hypersensitive to platinum compounds.[7-10]

Platinum-based agents are cytotoxic because they generate various types of DNA adduct, including interstrand cross-links (ICLs), intrastrand cross-links, and DNA-protein crosslinks, all of which block DNA replication and transcription.[11–14] However, cells have evolved repair mechanisms to resolve these lesions. The Fanconi anemia (FA) pathway is a major mechanism that repairs ICLs during DNA replication, and can be classified into 3 modules based on function: the FA core complex, which senses lesions and functions as a ubiquitin ligase for Fanconi anemia complementation group I (FANCI) and Fanconi anemia complementation group D2 (FANCD2); the ID2 complex comprising FANCI and FANCD2; and repair factors for ICLs, which include homologous recombination (HR) factors that are controlled by the mono-ubiquitinated ID2 complex.[13,14] Homologous recombination also plays crucial roles in other DNA repair processes, including single-strand DNA break repair.[15,16] Homologous recombination factors include BRCA1 and BRCA2 (also known as FANCS and FANCD1, respectively),[8,9,13,14,17,18] which could explain why BRCA1/2-deficient cancer cells are hypersensitive to platinum compounds.[7-10]

Poly(ADP-ribose) polymerase (PARP) inhibitors such as olaparib are an emerging class of antineoplastic agents that selectively damage BRCA1/2-deficient cancer cells.[19,20] Poly(ADP-ribose) polymerase 1 (PARP1), a target of PARP inhibitors, is involved in multiple DNA repair processes such as single-strand break repair; PARP inhibitors likely cause cytotoxicity by trapping PARP1 within damaged DNA.[21,22] Trapped PARP-DNA complexes could block replication fork progression, and the resulting lesions might be repaired by BRCA1/2-dependent HR. This might be why PARP inhibitors kill BRCA1/2-deficient cancer cells selectively.[19,20,23–26] In the clinic, PARP inhibitors are used to treat ovarian cancer either as a single agent or in combination with platinum compounds.[27,28]

MCM8 and MCM9 are paralogues of the MCM2-7 eukaryotic DNA replication helicase complex proteins. Originally, it was suggested that MCM8 and MCM9 regulate chromatin loading of MCM2-7 complexes[29–32]; however, accumulating evidence supports the view that MCM8 and MCM9 are involved in HR repair as a heterohexameric MCM8-9 complex.[33–35] Although the precise role of MCM8-9 in HR remains unclear, they could regulate either resection of DNA ends by MRN complexes[36] or processes downstream of Rad51 filament formation.[34,35] As expected from their involvement in HR, MCM8-9 play an important role in meiotic recombination in germline cells.[33,37] In addition, we previously reported that loss of MCM8-9 sensitizes chicken DT40 cells to ICL-inducers such as cisplatin and mitomycin C.[34] We also showed that MCM8-9 is required for HR-mediated DNA synthesis after fork breakage.[38] It is now thought that MCM8-9 plays a pivotal role in overcoming replication stress through HR-mediated long-tract gene conversion (LTGC) (see the Discussion for details).

Cancer cells undergo more replication stress than normal cells due to oncogenic hypergrowth stimuli.[39,40] Although the nature of the hyper-replication stress is still rather vague, it is likely that collision between DNA replication and transcription, both of which are stimulated by oncogenic stimuli, occurs more frequently in cancer cells.[40] Such collisions could stall replication forks and cause hyper-replication stress. At least some stalled forks will be converted to single-ended DNA double-stranded breaks, which are then repaired by HR.[41,42]

Taken together, the above findings suggest that inhibiting MCM8-9 could sensitize cancer cells to platinum compounds and PARP inhibitors. To provide evidence for this intriguing concept, we examined the effect of MCM8-9 inhibition on the sensitivity of cancer cells and nontransformed cells to cisplatin and olaparib. Also, we used nude mice bearing human tumor xenografts to examine the effect of MCM8-9 inhibition on cisplatin treatment. Taken together, the data strongly suggest that conceptual MCM8-9 inhibitors will be powerful cancer-specific chemosensitizers for platinum compounds and PARP inhibitors.

2 | MATERIALS AND METHODS

2.1 | Cells

HCT116, H1299, and U2OS cells were obtained from ATCC (Manassas, VA, USA). T98G cells were obtained from Dr. M. Enari, National Cancer Center Research Institute (Tokyo, Japan) in 2008. HeLa cells were obtained from JCRB Cell Bank (JCRB9004, authenticated by short tandem repeat PCR). Human telomere reverse-transcriptase (hTERT) RPE1 cells were purchased from Clontech (Mountain View, CA, USA). HFF2/T cells (human foreskin fibroblasts immortalized with hTERT) were established in-house.[43,44] These cells were cryopreserved in small aliquots and passaged (for less than 6 months after resuscitation) in DMEM supplemented with 8% FCS.

2.2 | Establishment of MCM9-knockout (KO) lines on HCT116 and hTERT-RPE1 backgrounds

A CRISPR-Cas9 vector was designed to target the third exon of the *MCM9* gene. The vector was constructed by introducing a hybridized oligonucleotide (caccgGCTTGTCTTCGTCTCCAACC and aaacGGTTGGAGACGAAGACAAGCc) into the *Bbs*I site of pX330 (Addgene plasmid #42230, a gift from Feng Zhang, Broad Institute of MIT and Harvard, Cambridge, MA, USA). A donor plasmid (pMK357), which harbors 1 kb homology arms around the target site, was also constructed for hygromycin selection. HCT116 cells were transfected with the CRISPR plasmid together with pMK357 using FuGene-HD (Promega, Madison, WI, USA), as previously described.[45] After selection in the presence of HygroGold (100 μg/mL; Invivogen, San Diego, CA, USA), isolated clones were further selected by genomic sequencing. To generate hTERT-RPE1 MCM9-KO cells, the same CRISPR plasmid was transfected in the absence of pMK357. Colonies formed in the absence of drug selection and isolated clones were selected by genomic sequencing. Modifications at the target locus are shown in Figure S1.

2.3 | Drugs

The following drugs were used: cisplatin (033-20091; Wako Pure Chemical Industries, Osaka, Japan), paclitaxel (169-18611; Wako

Pure Chemical Industries), and olaparib (MedChem Express, Monmouth Junction, NJ, USA; HY-10162). Paclitaxel and olaparib were dissolved in DMSO, and cisplatin was dissolved in saline.

2.4 | Immunoblot analysis and Abs

Immunoblot analysis was carried out as described previously.[46,47] Antibodies specific for the following were used: MCM8[34]; MCM9[34]; Pan-RAS (sc-166691; Santa Cruz Biotechnology, Dallas, TX, USA); p53 (OP43; Calbiochem, Dallas, TX, USA); Chk1 (sc-8408; Santa Cruz Biotechnology); phospho-Chk1 Ser345 (#2348S; Cell Signaling Technology, Danvers, MA, USA); Chk2 (#05-649; Upstate, Lake Placid, NY, USA); phospho-Chk2 Thr68 (#2661; Cell Signaling Technology); phospho-H2AX Ser139 (#07-164; Millipore, Burlington, MA, USA); phospho-ATM Ser1981 (#20772; Rockland, Limerick, PA, USA); and Rad51 (70-012; Bio Academia, Osaka, Japan). The following secondary Abs were used: HRP-rabbit anti-mouse IgG (H + L) (61-6520; Invitrogen, Carlsbad, CA, USA); HRP-goat anti-rabbit IgG (H + L) (65-6120; Invitrogen); Alexa 594-conjugated donkey anti-rabbit IgG (H + L) (A21207; Invitrogen); and Alexa 488-conjugated goat anti-mouse IgG (H + L) (A11029; Molecular Probes, Eugene, OR, USA).

2.5 | Immunofluorescence staining

After a brief rinse with cold PBS, cells were fixed for 15 minutes with 3.7% formaldehyde in PBS and permeabilized for 10 minutes with 0.1% Triton X-100 in PBS. After washing with PBS, cells were incubated for 1 hour at room temperature with appropriate primary Abs in PBS, followed by secondary Abs for 1 hour at room temperature, and then counterstained with DAPI. Cells were mounted in Fluoro-KEEPER Antifade Reagent (Nacalai Tesque, Kyoto, Japan) and analyzed using a Keyence (Osaka, Japan) BZ-X700 microscope. Image processing included haze reduction (Keyence) to optimize appearance.

2.6 | Growth inhibition assay

Cells were plated in 96-well plates (1.2×10^2 cells/well) and exposed to the indicated compounds (dissolved in DMSO or saline) 24 hours later. Either DMSO or saline alone was added to control cells (final concentration, 1%). After 48 hours, the medium was replaced with fresh medium and the cells were cultured for a further 120-144 hours. Subsequently, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium inner salt/phenazine methosulfate solution (CellTiter Aqueous Non-Radioactive Cell Proliferation Assay, #G5430; Promega, Madison, WI, USA) was added for 1 hour and the OD_{490} was measured to determine the number of viable cells. Percentage inhibition at each drug concentration was calculated, and the IC_{50} value for cell growth was calculated from the linear portion of the dose-response curve using regression analysis.

For the clonogenic assay, cells were transfected with the indicated siRNAs (see description below) for 24 hours. Cells were then replated on 12-well plates (at 400 cells/well) in complete medium containing the indicated concentration of each compound. After 7-9 days, colonies were stained with Giemsa solution (Wako Pure Chemical Industries). Percentage inhibition at each drug concentration was calculated, and the IC_{50} value for colony formation was calculated from the linear portion of the dose-response curve using regression analysis.

2.7 | Tumor studies in nude mice

All protocols for animal experiments were approved by the Animal Care and Use Committee of Kyushu University (Fukuoka, Japan; permit numbers: A25-022, A27-002, and A29-004). Four-week-old female BALB/c nu/nu nude mice were obtained from Kyudo (Saga, Japan) and inoculated (s.c. injection into the back) with 1×10^6 HCT116 WT or MCM9-KO cells mixed with Matrigel (50 μL cell suspension + 50 μL Matrigel; 356234, BD Biosciences, Franklin Lakes, NJ, USA). The size of the xenografted tumor was measured using digital calipers, and the volume was calculated using the following formula: tumor volume (mm^3) = length × $(width)^2 \times \pi/6$. Body weight and tumor size were measured 3 times per week. Once the tumor reached ~100 mm^3, mice were randomly divided into 2 groups: 1 group received control saline (vehicle) and the other received cisplatin (10 mg/kg). A single dose was given i.p.

2.8 | Small interfering RNA experiments

The following siRNA oligonucleotides were synthesized (sense strand sequences listed): MCM8-1 (5′-ACAAUAGAAUGUGAGCUUGUUCAdTdG-3′), MCM8-2 (5′-GGCAUACAGACAAAUACAUAAACdCdA-3′), p53-1 (5′-AGCAUCUUAUCCGAGUGGAAGGAdAdA-3′), p53-2 (5′-GAGGUUGGCUCUGACUGUACCACdCdA-3′), control siLuci (5′-GGUUCCUGGAACAAUUGCUUUUAdCdA-3′), and control siGFP (5′-ACCCUGAAGUUCAUCUGCACCACdCdG-3′). Cells (8×10^4 in 12-well plates) were transfected with 12 pmol siRNA duplexes using Lipofectamine RNAiMAX (Invitrogen).

2.9 | Data presentation and statistical analysis

Unless stated otherwise, quantitative data are represented as the mean ± SD of 3 or more independent experiments. For qualitative and semiquantitative data, a representative image from multiple independent experiments is shown; for all such figures, essentially the same results were obtained for each independent experiment.

The statistical significance of differences between 2 groups was analyzed using a two-tailed Student's *t* test. The statistical significance of the differences among more than 2 groups was analyzed using one-way ANOVA and Tukey's multiple comparison test. *P* values <.05 were considered statistically significant.

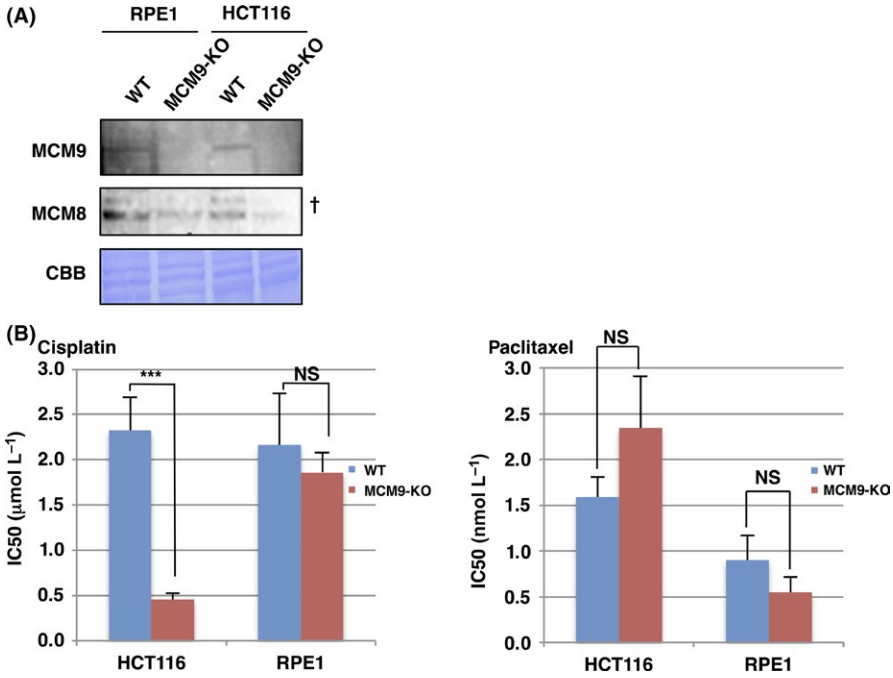


FIGURE 1 Knocking out MCM9 hypersensitizes colon cancer-derived HCT116 cells, but not untransformed human telomere reverse-transcriptase RPE1 cells, to cisplatin. A, Whole cell lysates were analyzed by SDS-PAGE, followed by immunoblotting with the indicated Abs. Proteins were stained with Coomassie Brilliant Blue (CBB) to check equal loading. †Nonspecific bands. B, The IC_{50} values for cisplatin and paclitaxel against the cell lines were calculated as follow. Cells were treated with the indicated compounds at various concentrations and percentage inhibition at each drug concentration was calculated. The IC_{50} value was then calculated from the linear portion of the dose-response curve using regression analysis. Data represent the mean ± SD from 5 independent experiments. ***$P < .005$. NS, not significant

3 | RESULTS

3.1 | Knocking out MCM9 hypersensitizes colon cancer-derived HCT116 cells, but not untransformed hTERT-RPE1 cells, to cisplatin

To examine the effect of MCM9 knockout on the sensitivity of cancer-derived and nontransformed human cells to cisplatin, we first utilized colon cancer-derived HCT116 cells and hTERT-immortalized normal human retinal pigment epithelial hTERT-RPE1 cells and established their respective MCM9-KO derivatives. Absence of MCM9 proteins from knockout cells was confirmed by immunoblotting (Figure 1A). We found that expression of MCM8 was also reduced in MCM9-KO cells (Figure 1A), a finding consistent with previous reports that MCM8 and MCM9 form a complex, and that depletion of either one affects the stability of the other.[34]

Next, we examined the effect of knocking out MCM9 on cisplatin sensitivity by estimating the IC_{50} (concentration yielding 50% inhibition) values for growth. The estimated IC_{50} values for cisplatin were ~2.3 μmol/L and 2.2 μmol/L in WT HCT116 and hTERT-RPE1 cells, respectively (Figure 1B). Knockout of MCM9 hypersensitized HCT116 cells to cisplatin (IC_{50}, 0.4 μmol/L) but had no significant effect on hTERT-RPE1 cells (Figure 1B). MCM9 knockout in either cell line had no effect on sensitivity to paclitaxel, a well-known antimitotic (Figure 1B). These data suggest that knocking out MCM8-9 selectively hypersensitizes cancer cells to cisplatin.

Generally speaking, p53 plays a crucial role in DNA damage-induced cell death. Therefore, we investigated the effect of p53 silencing on hypersensitization of HCT116 MCM9-KO cells to cisplatin; these cells retain WT p53. However, p53 silencing had no effect on the hypersensitivity of HCT116 MCM9-KO cells to cisplatin (Figure S2).

3.2 | MCM8 silencing by siRNAs hypersensitizes cancer and oncogene-transformed cells, but not untransformed human fibroblasts, to cisplatin

Next, we asked whether silencing MCM8 using siRNAs selectively hypersensitizes osteosarcoma-derived U2OS cells to cisplatin (compared with normal human HFF2/T fibroblasts). Silencing of MCM8 in both cell lines was confirmed by immunoblotting (Figure 2A). Also in these cell lines, expression of MCM9 was affected by MCM8 inhibition (Figure S3). We then evaluated colony formation after treatment of cells with different doses of cisplatin. As shown in Figure 2B, MCM8 knockdown significantly reduced colony formation by U2OS cells after cisplatin treatment. By contrast, MCM8 knockdown did not hypersensitize HFF2/T cells to cisplatin. Similar hypersensitization to cisplatin following MCM8 knockdown was also observed with H1299, HeLa, and T98G cancer-derived cells (Figure S4A,B).

We then asked whether siRNA-mediated silencing of MCM8 selectively hypersensitizes human fibroblasts transformed by the activated *KRAS* mutant G12V and the E7 of human papilloma virus type 16 (HPV16), which impairs RB, to cisplatin.[48] As expected, transformed HFF2/T E7/KRAS cells grew rapidly compared with parental HFF2/T cells (Figure 2C). Silencing of MCM8 was confirmed by immunoblotting (Figure 2D). Next, we examined colony formation after treatment with different doses of cisplatin. As shown in Figure 2E, MCM8 knockdown led to a significant reduction in colony formation by transformed cells after cisplatin treatment. By contrast, MCM8 knockdown did not hypersensitize parental HFF2/T cells. These results further support the idea that MCM8-9 inhibition selectively hypersensitizes cancer cells to cisplatin.

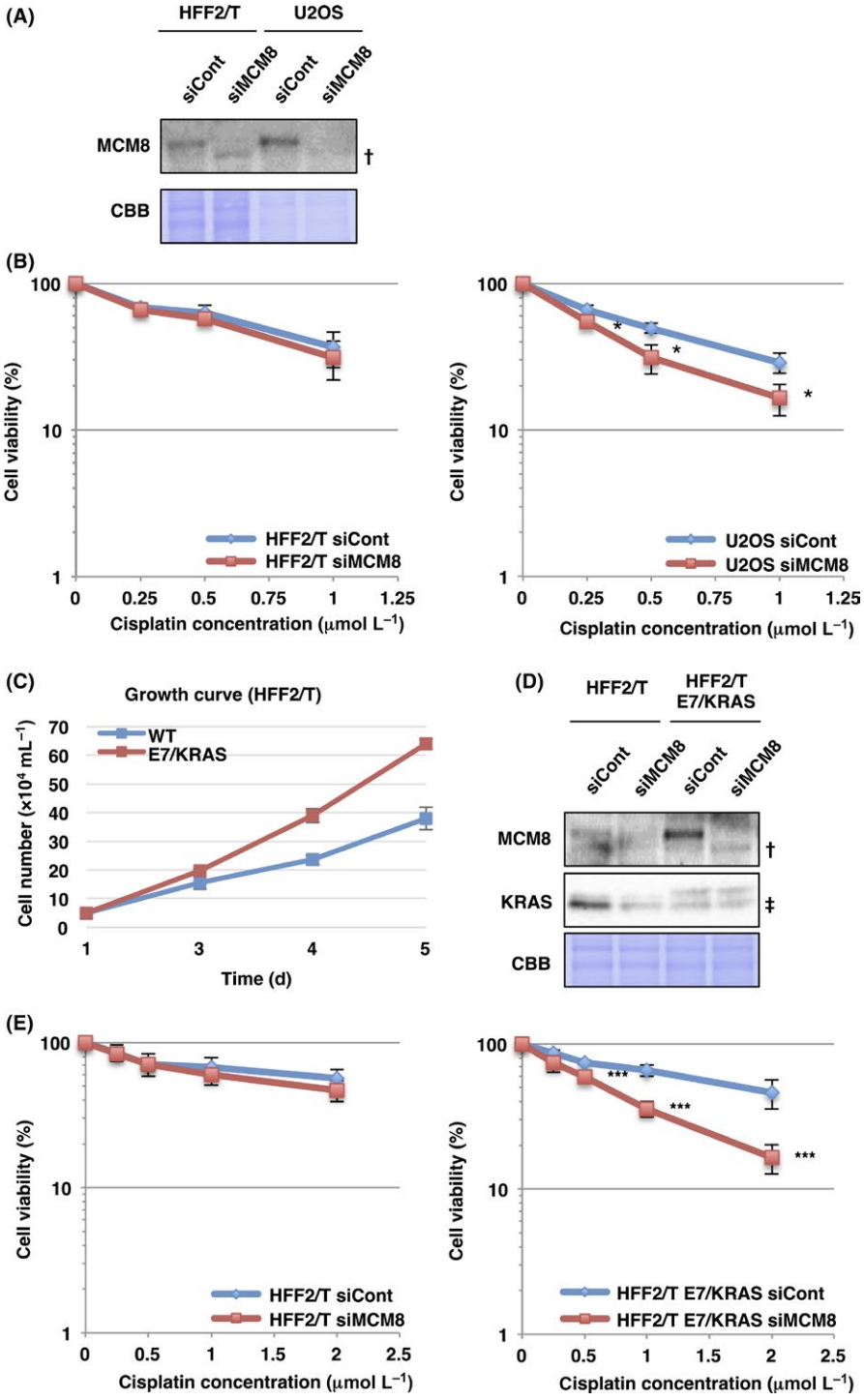
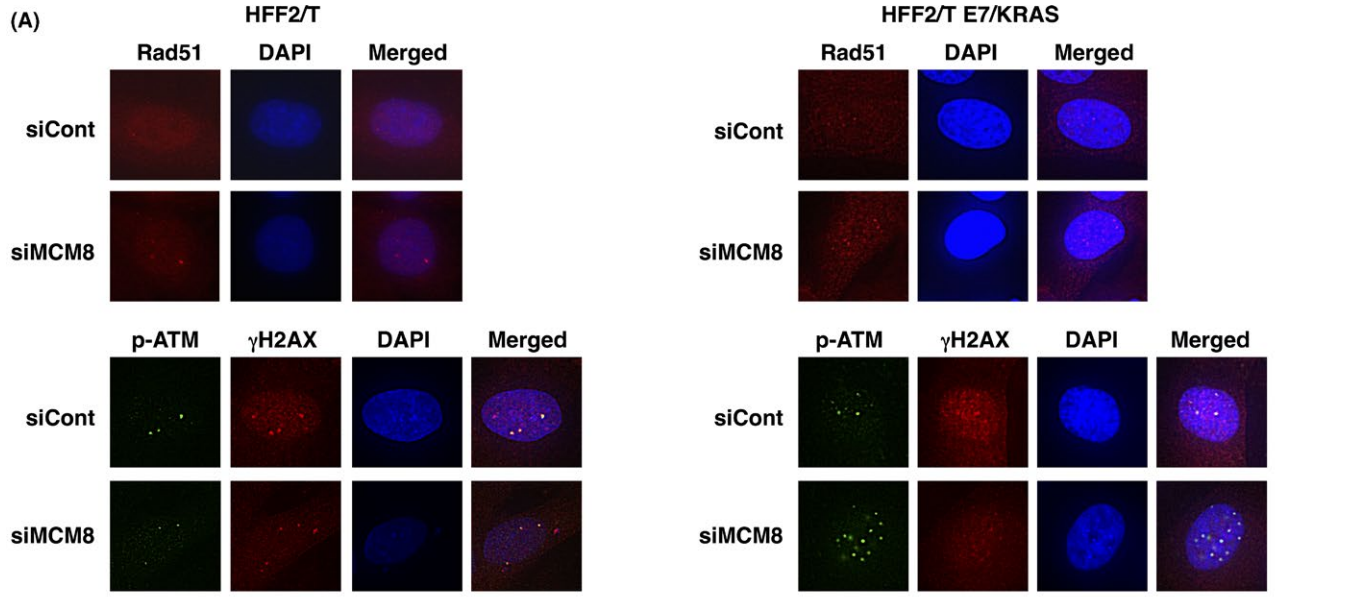
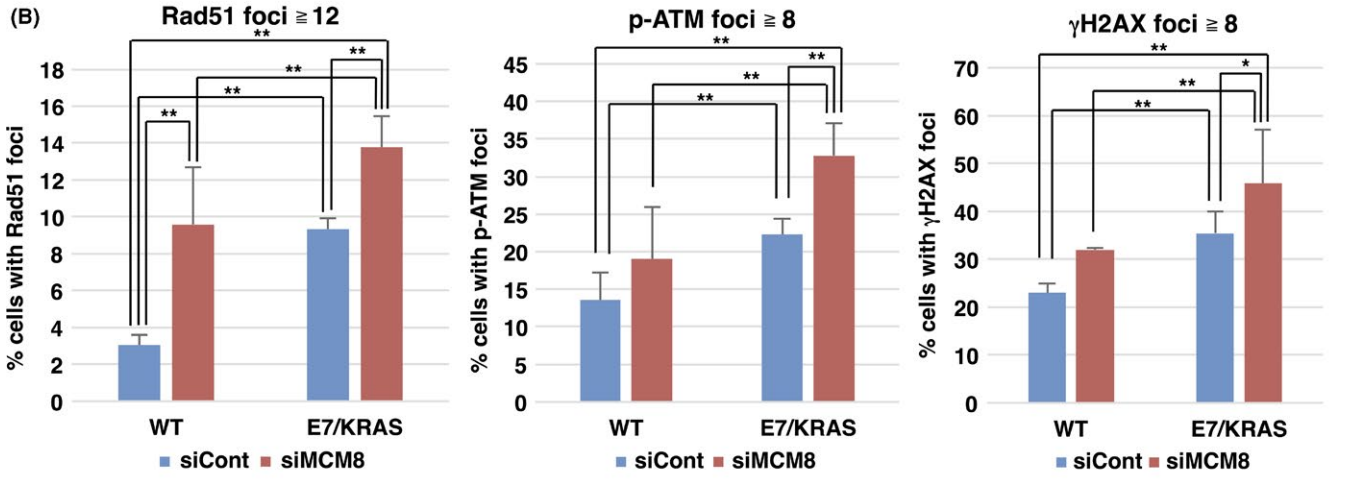


FIGURE 2 Silencing MCM8 using siRNAs hypersensitizes osteosarcoma-derived U2OS and KRAS G12V- and human papilloma virus type 16 E7-transformed HFF2/T cells, but not untransformed human fibroblast HFF2/T cells, to cisplatin. A, Cells were transfected with control (a mixture of siLuci and siGFP) (siCont) or MCM8-targeting (a mixture of siMCM8-1 and -2) (siMCM8) siRNAs for 24 hours and then subjected to immunoblotting with the indicated Abs. Proteins were stained with Coomassie Brilliant Blue (CBB) to check equal loading. †Nonspecific bands. B, Cells were transfected with the indicated siRNAs and subjected to a colony formation assay in the presence of the indicated drug concentrations. Data represent the mean ± SD from 4 independent experiments. $^{*}P < .05$. C, Growth of parental HFF2/T and HFF2/T E7/KRAS cells. Logarithmically growing cells were pelleted and resuspended at a concentration of 5×10^4/mL in fresh medium. Cells were then counted at the indicated days. Data represent the mean ± SD from 2 independent experiments. D, Cells were transfected with siCont or siMCM8 for 24 hours and subjected to immunoblotting with the indicated Abs. CBB staining was used to check equal loading. †Nonspecific bands. ‡Endogenous RAS proteins. E, Cells were transfected with the indicated siRNAs and subjected to a colony formation assay in the presence of the indicated drug concentrations. Data represent the mean ± SD from 6 independent experiments. $^{***}P < .005$

3.3 | Silencing MCM8 increases hyper-replication stress induced by oncogenic stimuli

As described above, cancer cells experience hyper-replication stress due to oncogenic hypergrowth stimuli.[39,40] In agreement with this, we found that RAS- and HPV16 E7-mediated transformation increased replication stress in human HFF2/T fibroblasts, as indicated by induction of multiple DNA damage responses. Thus, the number of Rad51, phospho-ATM Ser1981, and γH2AX foci in the cell nuclei increased (Figure 3A,B). Interestingly, all of these damage responses increased significantly upon MCM8 knockdown (Figure 3A,B). We then treated cells with cisplatin, and found that the levels of phospho-Chk1 Ser345 and phospho-Chk2 Thr68 increased after oncogenic transformation, and that the levels increased further following MCM8 knockdown (Figure 3C). These findings support the view that MCM8-9 function in HR-mediated repair of replication stress, and provide a rationale for MCM8-9 inhibition-mediated cancer-specific hypersensitization to cisplatin and olaparib.


(A)
HFF2/T
HFF2/T E7/KRAS
Rad51
DAPI
Merged
siCont
siMCM8
p-ATM
γH2AX
DAPI
Merged


(B)
Rad51 foci ≧ 12
p-ATM foci ≧ 8
γH2AX foci ≧ 8
% cells with Rad51 foci
% cells with p-ATM foci
% cells with γH2AX foci
WT
E7/KRAS
siCont
siMCM8

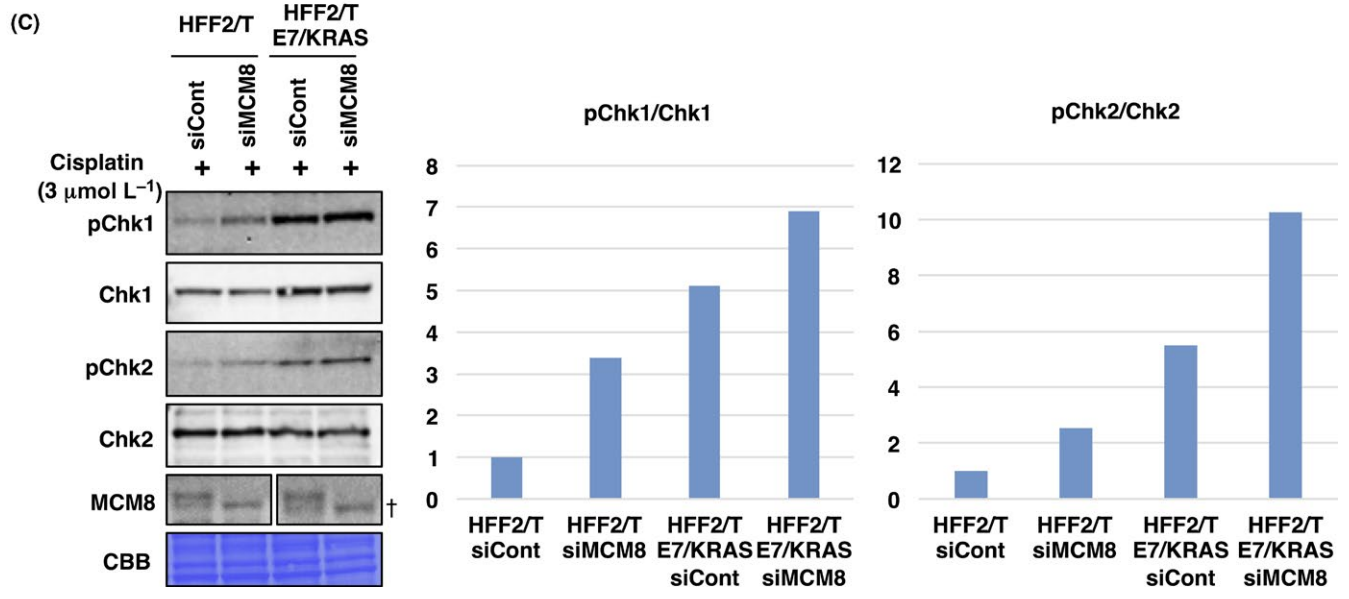

(C)
HFF2/T
HFF2/T E7/KRAS
siCont
siMCM8
Cisplatin (3 μmol L−1)
pChk1
Chk1
pChk2
Chk2
MCM8
CBB
pChk1/Chk1
pChk2/Chk2
HFF2/T siCont
HFF2/T siMCM8
HFF2/T E7/KRAS siCont
HFF2/T E7/KRAS siMCM8

FIGURE 3 Silencing MCM8 increases hyper-replication stress induced by KRAS G12V and human papilloma virus type 16 (HPV16) E7. A,B, Parental HFF2/T cells and cells expressing HPV16 E7 and KRAS G12V were transfected with control (a mixture of siLuci, and siGFP) (siCont) or MCM8-targeting (a mixture of siMCM8-1 and -2) (siMCM8) siRNAs for 24 hours, fixed, and immunostained with the indicated Abs. A, Representative images of Rad51, phospho-ATM Ser1981, and γH2AX foci in each cell type. B, The percentage of cells containing more than 12 Rad51 foci or more than 8 p-ATM, or γH2AX foci is shown. At least 100 randomly selected cells were analyzed for each experiment. Data represent the mean ± SD from 3 independent experiments. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. *$P < .05$. **$P < .01$. C, Cells were transfected with siCont or siMCM8 and treated simultaneously with 3 μmol/L cisplatin. After 24 hours, whole cell lysates were prepared and analyzed by immunoblotting with the indicated Abs. †Nonspecific bands. The signal intensities of total Chk1 or Chk2 and the phosphorylated (p)Chk1 or pChk2 were quantified, and the ratio of the pChk1/total Chk1 and the pChk2/total Chk2 was calculated. The graphs (right panels) show values relative to that for parental HFF2/T cells treated with control siRNAs set at 1

3.4 | Inhibiting MCM8-9 hypersensitizes cancer cells, but not untransformed cells, to olaparib

Poly(ADP-ribose) polymerase inhibitors induce replication fork stalling, which is (at least partly) repaired by HR to initiate fork restart.[21,22] Consequently, PARP inhibitors selectively damage BRCA1/2-deficient cancer cells.[19,20] We considered that a defect in MCM8-9 might have the same effect as depleting BRCA1/2; therefore, we investigated whether inhibiting MCM8-9 selectively sensitizes cancer cells to olaparib. First, we examined the effect of MCM9 knockout on the olaparib sensitivity of HCT116 and hTERT-RPE1 cells by estimating the IC_{50} values for growth. The estimated IC_{50} values were ~1.9 μmol/L and 6.9 μmol/L for WT HCT116 and RPE1 cells, respectively (Figure 4A). As for cisplatin, knockout of MCM9 hypersensitized

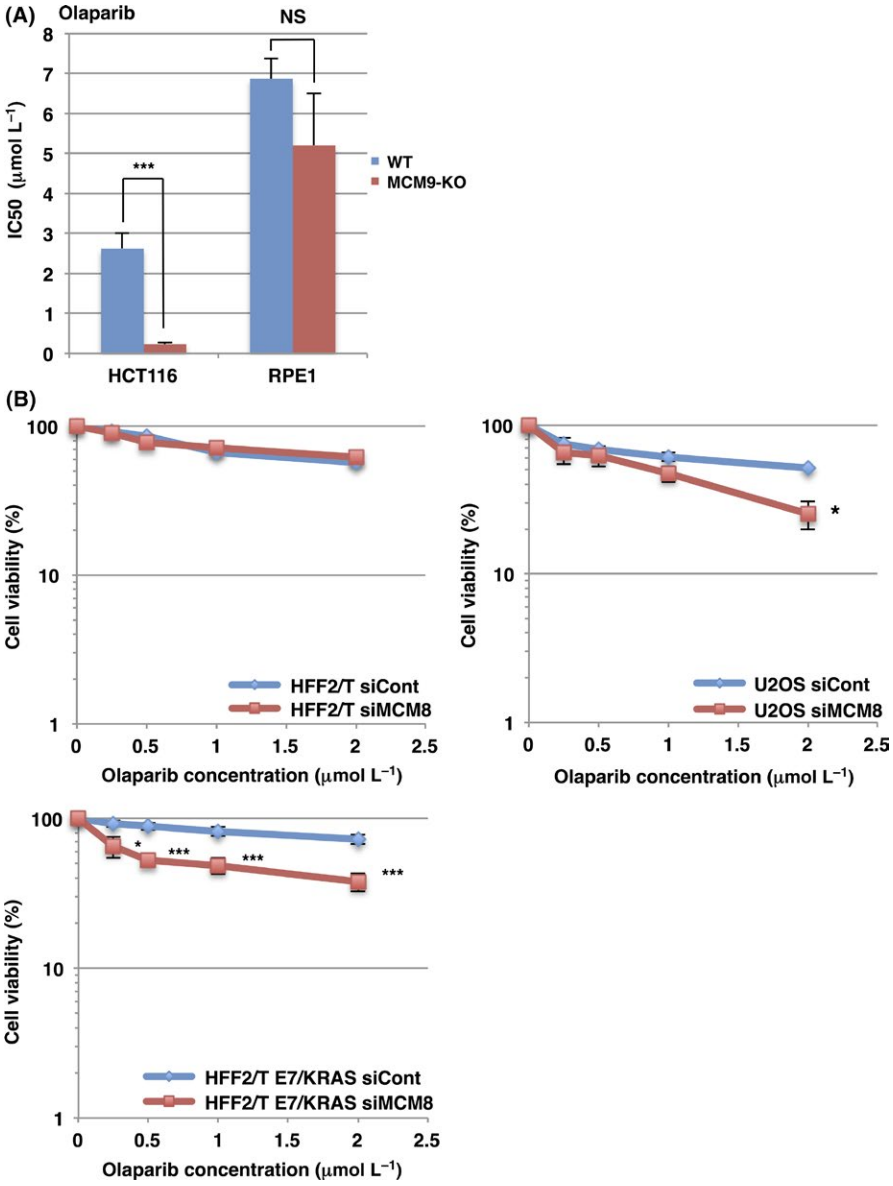


FIGURE 4 Knocking out MCM9 hypersensitizes cancer cells, but not untransformed cells, to olaparib. A, The IC_{50} values of olaparib against the indicated cell lines were calculated. Data represent the mean ± SD from 3 independent experiments. ***$P < .005$. NS, not significant. B, Cells were transfected with control (a mixture of siLuci, and siGFP) (siCont) or MCM8-targeting (a mixture of siMCM8-1 and -2) (siMCM8) siRNAs for 24 hours and subjected to a colony formation assay in the presence of the indicated concentrations of olaparib. Data represent the mean ± SD from 3 independent experiments. *$P < .05$. ***$P < .005$

HCT116 cells to olaparib (IC_{50}, 0.2 μmol/L), but had no significant effect on hTERT-RPE1 cells (Figure 4A). Thus, loss of MCM9 from HCT116 cells increases their sensitivity to olaparib by more than 10 times. Furthermore, we found that the sensitivity of MCM8-silenced U2OS and HFF2/T E7/KRAS cells, but not that of HFF2/T cells, to olaparib increased significantly (Figure 4B). Similar hypersensitization to olaparib following MCM8 knockdown was also observed with H1299 and HeLa cells (Figure S4A,C). Taken together, these results are consistent with the notion that inhibiting MCM8-9 selectively hypersensitizes cancer cells to olaparib as well as cisplatin.

3.5 | Knocking out MCM9 increases the sensitivity of HCT116 tumors to cisplatin

Finally, we examined whether depleting MCM9 hypersensitizes HCT116 tumors to cisplatin treatment. Accordingly, BALB/c nude mice bearing tumors derived from HCT116 WT or MCM9-KO cells received a single dose of control vehicle or cisplatin after the tumor reached a volume of 100 mm^3. There was no difference in the growth rate between HCT116 WT and MCM9-KO tumors (Figure 5A,B), indicating that MCM8-9 is not essential for tumor growth. This seems consistent with the fact that MCM9-KO mice grow normally.[37] We then examined the effect of cisplatin treatment. The growth of WT HCT116 cells was similar to that of control cells (Figure 5A). By contrast, the growth of HCT116 MCM9-KO cells was suppressed significantly by cisplatin (Figure 5B). These data provide further support to the idea that MCM8-9 inhibitors chemosensitize tumor cells to cisplatin.

4 | DISCUSSION

The data presented herein show that inhibiting MCM8-9 selectively hypersensitizes transformed cancer cells to the effects of cisplatin and olaparib, both of which induce replication fork blockade to cause cytotoxicity. The data also suggest that hypersensitivity is caused by oncogene-induced hyper-replication stress.

MCM8 and MCM9 operate during the HR-mediated DNA repair process.[33–35] As expected, MCM8-9 deficiency sensitizes cells to ICL-inducers such as cisplatin, and to a PARP inhibitor, olaparib. However, MCM8-9 deficiency does not sensitize cells to ionizing radiation (IR).[34-36] In general, IR damages DNA by generating 2 adjacent DNA ends, which are repaired during S phase by HR involving short-tract gene conversion between sister chromatids (or by nonhomologous end joining). However, ICLs and PARP inhibitors, which trap PARP1 on damaged DNA,[21,22] hamper replication fork progression during S phase; ultimately, the stalled forks will be converted to single-ended DNA breaks. To repair such regions by HR, LTGC between 2 distal DNA ends is required if a replication origin does not exist between the 2 regions. Therefore, the observed difference between sensitivity to replication stress and that to IR-induced double-stranded breaks suggests that MCM8-9 plays an important role in LTGC processes. Recently, we showed that this is indeed the case.[38]

Cancer cells are believed to be subject to more replication stress than normal cells due to oncogenic hypergrowth stimuli.[39,40] A major cause of hyper-replication stress could be increased replication fork stalling due to collisions between the DNA replication and transcription machineries.[40] Therefore, given that MCM8-9 plays a crucial role in the LTGC-mediated repair process during replication stress, it would seem reasonable that inhibiting MCM8-9 would selectively hypersensitize cancer cells to cisplatin and olaparib.

However, the protein complex most relevant to MCM8-9 is MCM2-7, a replicative helicase. Therefore, treatment with a specific MCM8-9 inhibitor could cross-inhibit the MCM2-7 helicase, which would be a problem as MCM2-7 is an essential replicative helicase; inhibition of this helicase will undoubtedly lead to cell growth inhibition. However, partial inhibition of MCM2-7 helicases does not hamper normal cellular replication under nonstressed conditions; rather, it sensitizes cells to replication stress.[49,50] This might be because excess MCM2-7 complexes are loaded onto chromatin; most

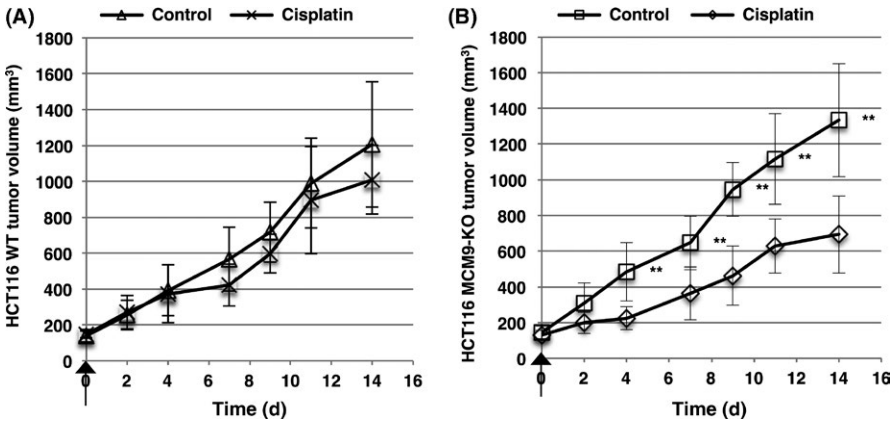


FIGURE 5 Knocking out MCM9 increases the sensitivity of HCT116 tumors to cisplatin. WT (A) and MCM9 knockout (KO) (B) HCT116 cells were inoculated s.c. into BALB/c nu/nu nude mice. Once the tumor reached ~100 mm^3, mice were randomly divided into 2 groups: 1 received control saline (vehicle) and the other received cisplatin (10 mg/kg). The drug was given once i.p. Mean tumor volumes (with SDs) are shown (n = 7). Arrow indicates the time of drug administration (day 0). $^{**}P < .01$

of these are dormant under nonstressed conditions but are reactivated as backup replication origins when replication is impeded.[51] Therefore, if a conceptual compound that strongly inhibits MCM8-9, but partially inhibits MCM2-7, is obtained, then it would (per se) be a candidate cancer-specific chemotherapeutic agent. Taken together, the data presented herein suggest that it is worth searching for new MCM8-9 inhibitors that can be developed as cancer-specific chemosensitizers for platinum compounds and PARP inhibitors. In the clinical setting, the secondary resistance of cancers against platinum compounds and PARP inhibitors arises. In this regard, it will be possible that conceptual MCM8-9 inhibitors can alleviate such resistance.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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