MCM8-9 complex promotes resection of double-strand break ends by MRE11-RAD50-NBS1 complex

Kyung Yong Lee1, Jun-Sub Im1, Etsuko Shibata1, Jonghoon Park1, Naofumi Handa2, Stephen C. Kowalczykowski2 & Anindya Dutta1

MCM8-9 complex is required for homologous recombination (HR)-mediated repair of double-strand breaks (DSBs). Here we report that MCM8-9 is required for DNA resection by MRN (MRE11-RAD50-NBS1) at DSBs to generate ssDNA. MCM8-9 interacts with MRN and is required for the nuclease activity and stable association of MRN with DSBs. The ATPase motifs of MCM8-9 are required for recruitment of MRE11 to foci of DNA damage. Homozygous deletion of the MCM9 found in various cancers sensitizes a cancer cell line to interstrand-crosslinking (ICL) agents. A cancer-derived point mutation or an SNP on MCM8 associated with premature ovarian failure (POF) diminishes the functional activity of MCM8. Therefore, the MCM8-9 complex facilitates DNA resection by the MRN complex during HR repair, genetic or epigenetic inactivation of MCM8 or MCM9 are seen in human cancers, and genetic inactivation of MCM8 may be the basis of a POF syndrome.
Double-strand break (DSB) repair is essential for the maintenance of DNA integrity. Deregulation of this process leads to significant genetic instability, which can result in the development of tumours. DSB repair systems are largely classified into homologous recombination (HR) and non-homologous end joining (NHEJ). In the first step of HR repair, MRN (MRE11-RAD50-NBS1) complex and its cofactors, NBS1 and MRE11, but also MCM8-9 (Fig. 3c, right). This result suggested that MCM8-9 might work during the initial DNA resection alongside the MRN complex. Therefore, we examined whether MRN and MCM8-9 physically associate with each other. Indeed, MCM9 immunoprecipitates contained not only its cofactors, NBS1 and MRE11, but also MCM8-9 (Fig. 3c, right). The co-immunoprecipitation was performed in the presence of the DNA intercalating chemical, ethidium bromide (EtBr), suggesting that the MCM8-9 and MRN did not interact via a bridging DNA molecule. Furthermore, MRE11 foci generated by binding to the I-SceI cut site was decreased in MCM8- or MCM9-depleted cells (Supplementary Fig. 1), RPA binding to the I-SceI cut site was decreased in cells depleted of MCM8 or MCM9 compared with control short interfering RNA (siRNA; siGL2)-transfected cells (Fig. 1c). To eliminate the possibility of off-target effects of siRNAs, we rescued cisplatin-induced RPA foci formation in siMCM8-transfected cells by stably expressing siRNA-resistant Flag-tagged MCM8 (Flag-MCM8r; Fig. 1d,e). H2AX phosphorylation on S139, and by inference the number of DNA breaks, was not affected by MCM8-9 depletion, suggesting that MCM8-9 specifically affected a step after DNA break formation.

To directly examine whether MCM8-9 is required for ssDNA formation, the generation of ssDNA at DSB sites was directly visualized by immunostaining with anti-BrdU (5-bromodeoxyuridine) antibody without denaturation of the double-stranded DNA after labelling of genomic DNA with BrdU in a previous cell cycle. Cisplatin increased the number of cells with BrdU signal, but this was decreased by depletion of MCM8 or MCM9 (Fig. 2a). Similarly, cisplatin treatment of mouse embryonic fibroblast (MEF) cells from Mcm9-null (XG/XG) mice produced fewer ssDNA-positive cells compared with wild-type (WT) MEFs (Fig. 2b). Cell cycle profile of the MEF cells from XG/XG mice was not changed by the 4-h cisplatin treatment, suggesting that the decrease in ssDNA formation could not be explained by a change in S phase progression in these cells (Supplementary Fig. 2). Next, we utilized a new assay for quantitatively measuring ssDNA at regions adjoining a specific DSB site induced by the restriction enzyme AssI in the estrogen receptor (ER)-AssI U2OS cells. The formation of ssDNA makes BsrG1 sites in the adjoining DNA resistant to digestion by that enzyme, thus allowing us to measure whether the end resection complex has digested past each of the three BsrG1 sites. DB induced by 4-hydroxytamoxifen (4-OHT) treatment resulted in more ssDNA at the BsrG1 site closer to AssI cut site, but at both this site and the next, depletion of MCM8 or MCM9 decreased the generation of ssDNA (Fig. 2c). Depletion of MRE11 also affected ssDNA formation at these sites, validating the assay. Therefore MCM8-9 is required for ssDNA formation at DSBs before HR.

MCM8-9 is required for MRN localization to HR repair sites.

To screen for the nuclease(s) requiring MCM8-9 activity for ssDNA formation, we first tested which nucleases were required for RPA focus formation after cisplatin treatment. The knockdown of MRE11, but not EXO1 and/or DNA2, suppressed cisplatin-induced RPA foci formation to the same extent as seen after MCM8-9 depletion (Fig. 3a,b and Supplementary Fig. 3a,b). This result suggested that MCM8-9 might work during the initial DNA resection alongside the MRN complex. Therefore, we examined whether MRN and MCM8-9 physically associate with each other. Indeed, MCM9 immunoprecipitates contained not only MCM8 but also MRE11, NBS1 and RAD50 (Fig. 3c, left), and conversely RAD50 immunoprecipitates contained not only its cofactors, NBS1 and MRE11, but also MCM8-9 (Fig. 3c, right). The co-immunoprecipitation was performed in the presence of the DNA intercalating chemical, ethidium bromide (EtBr), suggesting that the MCM8-9 and MRN did not interact via a bridging DNA molecule. Furthermore, MRE11 foci generated by cisplatin treatment co-localized with Flag-MCM8 foci (Fig. 3d). Chromatin immunoprecipitation (ChIP) assays on HeLa DR13-9 cells showed that all of the MRN components normally accumulated on the I-SceI cut site following the expression of

Results

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Deficiency of MCM8 or MCM9 impairs the recruitment of Rad51 on chromatin after DNA damage. DNA resection by the MRN complex, CtIP, EXO1 and DNA2, produces the ssDNA that has to be coated by RPA before subsequent Rad51 assembly. We thus measured the accumulation of RPA at DNA damage sites in MCM8- or MCM9-depleted cells. As reported previously, depletion of MCM8 decreases MCM9, but depletion of MCM9 does not deplete MCM8 (Fig. 1a). Even though levels of RPA70 were unchanged, there was a decrease in cisplatin-induced RPA foci formation on MCM8 or MCM9 depletion (Fig. 1a,b). HeLa DR13-9 cells contain a single I-SceI cut site integrated into their genome that is repaired by HR after cleavage by the I-SceI.

Although the expression level of I-SceI was not diminished in MCM8- or MCM9-depleted cells (Supplementary Fig. 1), RPA binding to the I-SceI cut site was decreased in cells depleted of MCM8 or MCM9 compared with control short interfering RNA (siRNA; siGL2)-transfected cells (Fig. 1c). To eliminate the possibility of off-target effects of siRNAs, we rescued cisplatin-induced RPA foci formation in siMCM8-transfected cells by stably expressing siRNA-resistant Flag-tagged MCM8 (Flag-MCM8r; Fig. 1d,e). H2AX phosphorylation on S139, and by inference the number of DNA breaks, was not affected by MCM8-9 depletion, suggesting that MCM8-9 specifically affected a step after DNA break formation.

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WT MCM9 partly restored HR efficiency to HeLa DR13-9 cells depleted of endogenous MCM9, whereas WA- or WB mutants of MCM9 did not (Fig. 4d).

Next, we examined whether the ATPase activity of MCM9 is necessary for MRE11 recruitment to DSBs. In ChIP experiments in HeLa DR13-9 cells, MRE11 recruitment to I-SceI cut sites was not rescued by MCM9 with mutations in WA or B motifs (Fig. 4e). The co-immunoprecipitation of MRE11 with MCM9 was also inhibited by the same mutations (Fig. 4f). Furthermore, in vitro nuclease assay on linearized pUC19 plasmid using purified HA-MCM9 from HeLa DR13-9 cells suggested that only MCM9 WT was associated with a strong nuclease activity (Fig. 4g).

MRE11 endonuclease initiates resection at DSBs before HR. We purified the MRN complex from U2OS cells stably expressing FLAG-NBS1 and tested its endonuclease activity on circular OXI74 ssDNA (Fig. 5a and Supplementary Fig. 7a). MCM8 knockdown decreased the endonuclease activity of

Figure 2 | ssDNA generation at DSB depends on MCM8 or MCM9. (a) ssDNA foci measured by BrdU staining without DNA denaturation and γH2AX immunofluorescence foci. Representative images on left and quantification of foci-positive cells on the right. Scale bar, 10 μm. ***P < 0.005, **P < 0.01; Student’s t-test. (b) ssDNA foci (BrdU foci) in MEFs with WT MCM9 (WT/WT) or with homozygous mutation for MCM9 (XG/XG) after exposure to cisplatin. Bottom: mean ± s.d. of triplicates. Scale bar, 10 μm. ***P < 0.005; Student’s t-test. (c) Quantitative measurement of DNA resection 4 h after addition of 4-OHT to ER-AsiSI U2OS cells. Percentage of ssDNA at indicated sites was measured by qPCR using the primer pairs indicated on the cartoon after digestion with BsrGI. Bottom: % of ssDNA at different sites. ***P < 0.005, *P < 0.05; Student’s t-test. All error bars represent s.d. of the mean from triplicates.

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Figure 3 | MCM8-9 are required for the localization of MRN complex on HR repair sites. (a,b) MCM8-9 and MRE11 required for forming RPA foci in cisplatin-treated cells. The western blot (a) and quantification of RPA70 foci-positive cells (b) after knockdown of indicated proteins in U2OS cells. ***P < 0.005; Student’s t-test. (c) MRN complex coimmunoprecipitates with MCM8-9. Endogenous MCM9 (left) or Rad50 (right) was immunoprecipitated (IP) from HEK293T cells using indicated antibodies in the presence of EtBr and immunoblotted for indicated proteins. (d) Co-localization of Flag-MCM8 and MRE11 in nuclear foci after exposure to cisplatin. Cells were pre-extracted for immunostaining. Scale bar, 10 μm. (e) Defect of MRN recruitment to I-SceI cut site in MCM8- or MCM9-depleted cells. ChIP assays were performed using indicated antibodies in HeLa DR13-9 cells 18 h after transfecting plasmid expressing I-SceI. Fold signal at cut site relative to site 2 kb upstream as described in Fig. 1c. ***P < 0.005, **P < 0.01, *P < 0.05; Student’s t-test. (f) Decrease of MRN foci-positive cells in MCM8- or MCM9-depleted cells. Representative images (left) and % of MRN foci-positive cells (right). Cells having over 20 foci > 0.5 μm diameter were counted as positive. Scale bar, 10 μm. ***P < 0.005, **P < 0.01; Student’s t-test. All error bars represent s.d. of the mean from triplicates. DAPI, 4',6'-diamidino-2-phenylindole; EV, empty vector; IgG, immunoglobulin G.
the immunoprecipitated MRN proteins, suggesting that human MCM8-9 is required for optimal nuclease activity of the MRN complex. Note that the nuclease activity of purified MRN complex was inhibited by MRE11 inhibitor, mirin (Supplementary Fig 7b). Thus, the nuclease activity in the MRN immunoprecipitate was mainly due to MRE11, although we cannot rule out the presence of other contaminating endonucleases.

Figure 4 | ATPase motif of MCM9 is essential for HR repair and the interaction with MRE11 protein. (a,b) Mutation on ATPase motif of MCM8-9 decreases RPA (top) or MRE11 (bottom) foci formation. U2OS cells supported by Walker A- (WA) or Walker B (WB) mutants of MCM8 (a) or MCM9 (b) were treated with cisplatin after knockdown of the endogenous protein, and foci-positive cells were counted as described previously. ***P<0.005, **P<0.01, *P<0.05; Student’s t-test. (c) WA- or WB mutant of MCM9 cannot restore resistance to cisplatin after knockdown of endogenous MCM9. Cell viability was measured by colony count at day 5 after cisplatin treatment. ***P<0.005; Student’s t-test. (d) WA- or WB mutant MCM9 cannot rescue HR. HR assays were performed in HeLa DR13-9 cells having stable expression of siRNA-resistant MCM9. HR efficiency was measured by normalizing the percentage of GFP-positive cells of each sample to that of the siGL2-treated cells. ***P<0.005; Student’s t-test. (e) WA- or WB mutant MCM9 cannot recruit MRE11 to I-SceI cut site. ChIP was done using HeLa DR13-9 cells having stable expression of siRNA-resistant MCM9 after knockdown of endogenous MCM9. Signal at cut site expressed relative to -2 kb site. **P<0.01, *P<0.05; Student’s t-test. (f) WA- or WB mutant MCM9 does not co-immunoprecipitate MRE11 from HEK293T cells transfected by the indicated plasmids expressing MCM9. (g) Decrease in nuclease associated with WA- or WB mutant MCM9. DNA products visualized after in vitro nuclease assay for 90 min with epitope-tagged MCM9 immunoprecipitated (IP) from cells transfected with indicated plasmids and siRNAs as described in Methods section. Cells were treated with 40 μM cisplatin for 4 h before harvest. All error bars represent s.d. of the mean from triplicates. EV, empty vector; WT, wild type.
Next, we purified recombinant *Xenopus* MCM8-MCM9 WT (WT/WT) from baculovirus-infected insect cells to investigate whether MCM8-9 can stimulate MRE11 endonuclease *in vitro* (Fig. 5b). MCM8 WT MCM9 WA mutant (WT/WA) complexes were used as inactive controls. We were thwarted from doing the experiment by a nuclease that was associated with the purified WT/WT MCM8-9 but not with the inactive WT/WA MCM8-9 (Fig. 5c). Since the WA mutation of human MCM9 decreased its association with human MRE11 (Fig. 4f), we wondered whether insect MRE11 was co-purifying with the XI-WT/WT MCM8-9 complex. Remarkably, a 65-kDa protein, corresponding in size to *Drosophila* MRE11 and recognized by anti-MRE11 antibody, was associated with WT/WT but not WT/ WA MCM8-9 complex (Fig. 5d, the B lanes). Anti-MRE11 antibody immunodepleted the 65-kDa protein (Fig. 5d, the A and I lanes) and decreased the nuclease activity associated with MCM8-9 (Fig. 5e). Several controls showed that the WA mutation of MCM9 did not decrease MCM8-9 complex formation (Supplementary Fig. 8), but decreased the ATPase activity of MCM8-9 (Supplementary Fig. 9), and that the MRE11 antibody decreased the nuclease associated with the recombinant XI-MCM8-9 without decreasing the amount of MCM8-9 proteins (Supplementary Fig. 10).

These results suggest that ATPase activity of MCM8-9 complex is required for ssDNA generation at DSBs as well as optimal nuclease activity of the MRN. Also, ATP binding and hydrolysis by the MCM9 in the MCM8-9 complex is essential for maximal association with Mre11 and for the recruitment of MRN to DNA damage sites.

**Functional inactivation of MCM9 in cancers.** Human cancers frequently show homo- and heterozygous deletions or translocations on 6q22.31, the genomic region containing the MCM9 gene.\(^{27-29}\). Copy-number variation studies in cancer genomes collated at CBioPortal\(^3\), and show that 6–7% of prostate cancers and salivary adenoid cystic carcinomas, and a smaller fraction of other cancers have homozygous deletion of MCM9 (examples listed in Table 1). Several cancer cell lines, including a non-small cell lung cancer cell line, NCI-H2291, also have a homozygous deletion of the MCM9 locus. To test whether the cancer-specific loss of MCM9 affected HR repair, we compared the ability of NCI-

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**Figure 5 | ATPase activity of MCM9 is essential for the function of MRN nuclease.** (a) Decrease of MRN endonuclease activity in MCM8-9-depleted cells. In *in vitro* endonuclease assay using MRN purified by anti-FLAG from U2OS cells stably expressing FLAG-NBS1, ØX174 ssDNA substrate was incubated for indicated times. Top: EtBr stain of reaction products shows the substrate C, circular ØX174 and the product L, linearized ØX174. Bottom: quantification of linearized ssDNA with ImageJ software, normalized to the level in the 0-min lane. \(***P<0.005, *P<0.05;\) Student’s t-test. (b) Silver stain of purified recombinant *Xenopus* MCM8-9 complex. WT, wild type-MCM8 and -MCM9; WA, WA mutant MCM9. (c) WT/WT MCM8-9 has more nuclease activity than WT/WA MCM8-9 complex. WT, wild type-MCM8 and -MCM9; WA, WA mutant MCM9. (d) Immunodepletion of MRE11, as detected by immunoblot, from purified recombinant MCM8-9. Immunodepletion was done by incubating anti-human MRE11 antibody with indicated recombinant proteins. B, before immunodepletion; A, after immunodepletion; I, the total immunoprecipitate. (e) Reduced nuclease activity of WT/WT MCM8-9 after immunodepletion of MRE11 (A), compared with that before immunodepletion (B). Amount of full-length linear DNA remaining was quantified after 60 min of an *in vitro* nuclease assay with 5 nM of BSA or purified MCM8-9 before or after immunodepletion of MRE11. The y axis shows the ratio of the residual substrate relative to that at the 0-min point. \(***P<0.005, *P<0.05;\) Student’s t-test. All error bars represent s.d. of the mean from triplicates.
H2291 and NCI-H1299, a control non-small cell lung cancer cell line, to cope with cisplatin-induced DNA damage. Although protein levels of MCM8 and other HR repair factors were similar, MCM9 protein was not detected in NCI-H2291 (Fig. 6a). The NCI-H2291 cells showed fewer cells positive for RPA and MRE11 foci on knockdown of endogenous MCM8 (Fig. 7a, lower panel). Thus, deletion of MCM9 produces a functional defect of HR repair in this cancer cell line. In addition, we found that several prostate cancer cell lines do not have deletions in MCM9, but express lower levels of the MCM9 protein compared with HEK293T cells (Fig. 6f). The cisplatin resistance of these prostate cancer cell lines was remarkably correlated to the amount of MCM9 protein expressed (Fig. 6g and Supplementary Fig. 12). Therefore, epigenetic suppression of MCM9 could also predispose cancer cells to cisplatin sensitivity.

**Functional inactivation of MCM8 by mutation in cancer or POF.**

Finally, we turned to a lung squamous cell carcinoma in CBioPortal, with a point mutation that changed proline 456 in the WA motif of MCM8 to alanine (Fig. 7a, the upper panel). Clonogenic assays in a cell line stably expressing siRNA-resistant MCM8 P456A showed that this mutation inactivated MCM8 because there was a decrease of cisplatin-induced RPA70- and MRE11 foci on knockdown of endogenous MCM8 (Fig. 7a, lower panel). Thus, genetic and epigenetic inactivation of MCM8-9 is seen in diverse human cancers, and such inactivation is associated with sensitivity to therapy by ICL agents.

Genome-wide association studies (GWAS) identified an SNP on MCM8 associated with an early age of natural menopause (a POF syndrome). MCM8 E341K was not able to rescue the RPA70- and MRE11 foci formation seen after depletion of endogenous MCM8 (Fig. 7b,c) nor could it fully restore the sensitivity of the MCM8-depleted cells to cisplatin (Fig. 7d). Consistent with a previous result, the naturally occurring SNP of MCM8 associated with POF impairs the function of MCM8 in HR repair. Since HR repair is an essential function of meiosis, mutational inactivation of MCM8 may explain the genetic basis of this POF syndrome.

**Discussion**

DNA end resection is more pronounced in HR than in classical NHEJ. In this study, we show that MCM8-9 is required for ssDNA generation at DSBs using the following three independent approaches: (a) detection of RPA70, (b) exposure of BrdU-labelled DNA and (c) quantitative PCR (qPCR)-based assay to measure ssDNA adjoining a DSB (Figs 1 and 2). Several DNA nucleases have been implicated in the resection: MRN complex and CIP for the initial resection/EXO1 and DNA2 for the longer stretches of ssDNA. It has also been suggested that MRE11 endonuclease initiates resection followed by bidirectional exonuclease activity of MRE11 and EXO1 to generate ssDNA for HR. In our hands, EXO1 and DNA2 appear dispensable for RPA focus formation after cisplatin treatment, in contrast to the requirement of MRE11 (Fig. 3b and Supplementary Fig. 3b), perhaps because the secondary and extensive DNA resection attributed to EXO1 and DNA2 is slower than that caused by MRE11, and is not necessary for forming RPA foci. This does not mean that EXO1 and DNA2 are totally dispensable for resection in vivo. However, the parallel effects of MCM8-9 and MRE11 depletion on RPA focus formation is consistent with our suggestion that MCM8-9 is particularly involved in the initial resection by MRE11.

MCM8-9 recruits or promotes the stable association of MRN to the DSB (Fig. 3), without much effect on CIP recruitment. This suggests that CIP can be recruited to DSBs independent of MRN, which is in line with a previous report. Also, since the MRN complex directly binds to DNA ends in vitro, these results suggest either that MCM8-9 is required in cells on top of the direct DNA binding to recruit MRN, or that MCM8-9 prevents the detachment of active MRN after it has been recruited. MCM8-9 has highly conserved ATPase motifs and shows a helicase activity in vitro. We show that mutation of its ATPase motif causes loss of interaction with MRN complex, increase of cellular sensitivity to cisplatin as well as decrease of HR efficiency (Figs 4 and 5). If anything, the mutant forms of MCM8 appear to have a dominant-negative effect on checkpoint activation (an indirect measure of ssDNA formation), HR and cell survival after cisplatin treatment (Supplementary Fig. 5 and Fig. 4c,d). The WA- or WB mutants of MCM8 most likely associate with endogenous MCM8 or other endogenous proteins to inactivate them further than seen after simple depletion of MCM9.

MCM8-9 ATPase could be a new therapeutic target whose inhibition would impair HR and augment the efficacy of ICL-inducing agents during chemotherapy. However, it remains to be elucidated how ATPase activity of MCM8-9 promotes the functional activity of MRN. Considering that DNA helicases utilize ATP hydrolysis as an energy source, MCM8-9 may function as a helicase in its support of MRN. Alternatively, the physical association of MCM9 with MRE11 is impaired by the mutations in the WA or B motifs of MCM9 (Fig. 4f), suggesting that ATP binding or hydrolysis is important for the protein–protein interaction between these two complexes.

### Table 1 | A list of some of the cancers reported to have homozygous deletion of MCM9 locus from copy-number variation (CNV) experiments collated in CBioPortal.

| Cancers                          | Case ID                      |
|----------------------------------|------------------------------|
| Breast invasive carcinoma        | TCGA-AR-A0TW                 |
| Glioblastoma                     | TCGA-AR-A1AU                 |
| Kidney renal clear cell carcinoma| TCGA-CZ-5460                 |
| Ovarian serous cystadenocarcinoma| TCGA-24-2267                 |
| Prostate adenocarcinoma          | PCA019                       |
| Bladder urothelial carcinoma     | TCGA-H4-A2HQ                 |
| Head and neck squamous cell carcinoma| TCGA-CQ-5329               |
| Prostate adenocarcinoma          | TCGA-CH-5748                 |
| Skin cutaneous melanoma          | TCGA-D9-A149                 |
| **Cancers with homozygous deletion of MCM9 locus** | **Case ID** |
| Bladder urothelial carcinoma     | TCGA-H4-A2HQ                 |
| Head and neck squamous cell carcinoma| TCGA-CQ-5329               |
| Prostate adenocarcinoma          | TCGA-CH-5748                 |
| Skin cutaneous melanoma          | TCGA-D9-A149                 |
Figure 6 | Functional inactivation of MCM9 in cancers. (a) Absence of MCM9 protein in NCI-H2291. Immunoblot of indicated proteins in lysates of NCI-H2291 and NCI-H1299 (control cell line). (b) Decrease of cisplatin-induced RPA70- or Mre11 foci-positive cells in NCI-H2291. **P < 0.005; Student’s t-test. (c) Decrease of HR efficiency in NCI-H2291. HR assay was performed by transient transfection of DSB recombination reporter and I-SceI expression plasmids as described in Methods section. HR efficiency was calculated by normalizing the percentage of GFP-positive cells to transfection efficiency in each cell line. ***P < 0.005; Student’s t-test. (d) Transient expression of ectopic HA-MCM9 (WT) in NCI-H2291. (e) Restoration of relative resistance of NCI-H2291 to cisplatin by overexpression of MCM9. Cell viability was measured by clonogenic assay as described in Methods section. Top: representative wells. Bottom: quantification of viable cells. **P < 0.01; Student’s t-test. (f) Protein expression of MCM9 in prostate cancer cells. Top: amount of MCM9 protein in each cancer cell line measured by immunoblotting. All lanes were in the same blot and exposed similarly. Bottom: MCM9 signal quantified with ImageJ software, normalized to α-tubulin and expressed relative to 293T. (g) Correlation of MCM9 levels to IC75 to cisplatin in indicated cancer cell lines (also see viability curves measured by MTT assay in Supplementary Fig. 12). All error bars represent s.d. of the mean from triplicates. EV, empty vector.

Although MCM8-9 has been shown to associate weakly with RAD51 (ref. 17), the results we present here argue against that being the primary function of MCM8-9 in the loading of RAD51. Instead, MCM8-9 is clearly involved in preparing the ssDNA substrate that eventually loads RAD51.

Impaired HR makes cancers more sensitive to ICL-inducing agents. We show that a naturally occurring homozygous deletion of the MCM9 sensitizes a cancer cell line to ICL reagents and leads to the HR defect (Fig. 6). The expression level of the MCM9 protein is correlated to the cisplatin resistance of some prostate cancer cell lines (Fig. 6f,g) and a cancer-derived mutation of MCM8 inactivated MCM8 (Fig. 7a). Thus, the deletion, point mutation or reduced expression of MCM8 or MCM9 in cancers that we report here should be tested in patients for predicting a cancer’s sensitivity to ICL-inducing agents such as cisplatin or mitomycin C. As a promoter of HR, MCM8 or MCM9 may serve as a bona fide tumour suppressor similar to other genes important for HR, BRCA1 and BRCA2. A cancer-derived point mutation and a naturally occurring SNP on MCM8 associated with POF diminish the functional activity of MCM8 (Fig. 7). Together, with a very recent study that appeared while this paper was under review31, our results explain how the SNP in MCM8 impairs HR, an essential function in the germ line, and thus could lead to a genetically determined POF syndrome.

Methods
Cell lines and siRNAs. U2OS, ER-AsSi U2OS, HeLa DR13-9 and HEK293T cells were grown in Dulbecco’s modified Eagle’s medium (Thermo Scientific) with 10%
fetal bovine serum (FBS; Sigma-Aldrich) and penicillin/streptomycin (1%, Gibco). LNCaP, PC-3 and NCI-H1299 cells were grown in RPMI 1640 (Gibco) supplemented with 10% FBS and 1% penicillin/streptomycin. WPE1-NB26 cells were maintained in kera-

Establishment of stable cell lines. The siRNA-resistant WT or mutant of MCM8 and MCM9 was inserted into retrovirus vector pBabe-puro and pLHCX (Clon-tech), respectively. Retroviral vectors, together with retroviral packaging vector, were transfected into 293T cells using Lipofectamin 2000 (Invitrogen) according to the manufacturer’s instruction. At 48 h after transfection, viral culture supernatants were harvested and filtered through a 0.45-μm filter, and added to the U2OS or HeLa DR13-9 cells in the presence of 8 μg ml⁻¹ polybrene (Sigma-Aldrich). After 48 h of infection, drug selection was carried out with either 100 μg ml⁻¹ hygromycin B (Sigma-Aldrich) or 2 μg ml⁻¹ puromycin (Sigma-Aldrich) to select retrovirus-infected cells over a 10-day period.

Immunoblotting and antibodies. For immunoprecipitation, cells were washed with PBS once and lysed by lysis buffer (20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1% Nonidet P-40 (NP-40), 5% glycerol, 1 mM EDTA, 1 mM MgCl₂, 1 mM ATP, 1 mM diithiothreitol (DTT), 10 mM NaF, 1 mM Na₃VO₄ and protease inhibitors). After sonication and centrifugation at 21,130 × g for 30 min, 3 mg of lysates was incubated with indicated antibodies and pulled down with protein G-conjugated agarose beads (GE Healthcare) in the presence of EBlR (10 μg ml⁻¹). For immunoblotting, 50–70 μg of protein was subjected to SDS–polyacrylamide gel electrophoresis analysis. Antibodies for this study were as follows: MCM8 and MCM9 antibodies were raised in rabbits against the N- and C-terminal 100 amino acids of MCM8 and MCM9 proteins, respectively. Anti-MCM8 (Immunoblot (IB), 1:100) and anti-MCM9 (IB, 1:500); Immunoprecipitation (IP), 12 μl per reaction); anti-RPA70 (IB, 1:1,000; Immunofluorescence (IF), 1:100; NA13; Calbiochem); anti-MRE11 (IB, 1:1,000); anti-Rad50 (IB, 1:1,000; ab96488; Abcam); anti-p-CHK1 (Ser317) (IB, 1:1,000; 2344S); anti-FLAG (IF, 1:1,000; F1804; Sigma); anti-α-tubulin (IB, 1:1,000; sc-2577; Cell Signaling Technology); anti-α-ME11 (IB,
Immunostaining presented in the main paper are provided in Supplementary Fig. 13. Uncropped images of immunoblots used as loading control in most immunoblots. Cells were washed three times with PBS and incubated with either Alexa Fluor 555 anti-rabbit (1:1,000, A21429; Life Technologies) or Alexa Fluor 488 anti-mouse (1:500, A11029; Life Technologies) immunoglobulin G secondary antibody. Cells were mounted with a solution containing 4,6-diamidino-2-phenylindole (Vector Laboratories, Inc.) before being examined under a microscope. To detect ssDNA, U2OS cells were incubated with 10 μM of BrdU for 24 h before the first transfection of siRNA, and BrdU was maintained until 48 h after harvest to confirm transient expression. MEFs were transfected with 10 μM BrdU for 48 h. In most experiments, 40 μm clastatin was added for 4 h before harvest and pre-extraction was performed using 0.5% Triton X-100 in PBS for 1 min only for BrdU staining (1:100, 55827; DB Pharmingen). A Zeiss AxiosObserver equipped with an EC Plan-Neofluar (80/0.4) oil and Axiovision software were used to obtain and analyse images, respectively. Cells having > 20 foci per cell were counted. Brightness and contrast of obtained images were adjusted using Photoshop 7.0 (Adobe).

ChiP assay. HeLa DR13-9 cells stably expressing MCM9 (WT or mutant in WA/WB motifs) were fixed with 1% formaldehyde for 10 min at room temperature and incubated in 100 mM NaCl, 1% Triton X-100, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS and 0.1% sodium deoxycholate, washed once with PBS and resuspended in 50 ml of lysis buffer (10 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0), 0.1% SDS and protease inhibitor cocktail (Roche)). The lysates were collected by centrifugation at 13,000 r.p.m. for 10 min at 4°C. The cleared lysates were mixed with beads (Qiagen) for 1 h and the beads were washed with lysis buffer supplemented with 20 mM imidazole. Bound proteins were eluted with lysis buffer supplemented with 200 mM imidazole, and then diluted fivefold with dilution buffer (20 mM Tris- HCl (pH 7.4), 0.1% NP-40, 10 mM MgCl2, 5 mM 2-mercaptoethanol and 10% glycerol). The eluted proteins were applied to Hitrap Heparin HP column (Amersham Pharmacia Biotech) in the fast protein liquid chromatography (FPLC), and 1 ml fractions were collected in elution buffer with a linear gradient from 50 mM to 1 M NaCl. The peak fractions were pooled and desalted by a PD-10 desalting column (GE Healthcare Life Sciences). These were next applied to a Hitrap Q HP column (Amersham Pharmacia Biotech) in the FPLC, and 1 ml fractions were collected in elution buffer with a linear gradient from 100 mM to 500 mM NaCl. Final peak fractions were concentrated by using Amicon Ultra Centrifugal filters (Millipore), and elution buffer was changed into storage buffer (20 mM Tris-HCl (pH 7.4), 100 mM NaCl, 0.01% Triton X-100, 10% glycerol and 1 mM DTT) by a PD-10 desalting column. To check the purity in each step of purifications, small aliquots of each fraction were run on a 8% SDS-PAGE gel and silver-stained using a Silver Stain Kit (Pierce).

Immunodepletion of insect MRRE1 from purified MCMC8-9. The protein G-conjugated agarose beads conjugated with anti-MRE11 antibody were incubated with purified proteins at 4°C overnight and separated from supernatant. One-fifth of the input protein before immunodepletion (A) or after immunodepletion (B) and the total immunoprecipitates (I) were loaded on 8% SDS-PAGE gel and detected by anti-human MRE11 antibody in the western blot technique.

In vitro nuclease assay. To purify FLAG-tagged NBS1 or MCM9 protein, we used U2OS and HeLa DR13-9 cell lines that stably express FLAG-NBS1 and HA-MCM9, respectively. Cells were transfected with siGL2, siMCM8 or siMCM9 for 48 h and each of the lysates were obtained by the same procedures as used for immunoprecipitation assays. Ten micromoles of anti-FLAG M2 affinity gel (A2220, Sigma) or EZview Red Anti-HA Affinity beads (6E779, Sigma) was incubated with 1.5 or 3 mg of lysate overnight at 4°C, and washed with lysis buffer four times and then with elution buffer (62.5 mM HEPES (pH 7.4), 62.5 mM KCl, 5% glycerol, 1 mM DTT and 50 μg ml−1 BSA) accompanied by rotation for 5 min each wash. Finally, proteins were eluted by incubation with 20 μl of elution buffer supplemented with 150 μg ml−1 FLAG or HA peptide (sigma). The in vitro nuclease assay was done as previously published with slight modifications3,6, pUC19 (1.88 or 3.75 nm) plasmid linearized by HindIII was used with the indicated amount of purified MCMC8-9 or purified MRN (FLAG-NBS1) in reaction buffer (20 mM HEPES (pH 7.5), 0.1 mM DTT, 5 mM MnCl2, 2 mM ATP, 0.1 mg ml−1 BSA and 0.05% Triton X-100) for 15 or 30 min at 37°C. Alternatively, 100 ng of circular OX174 ssDNA virion DNA (New England Biolabs) was mixed with purified MRN (FLAG-NBS1) in reaction buffer (30 mM Tris-HCl (pH 7.5), 1 mM DTT, 25 mM KCl, 200 ng acetylated BSA, 0.4% DMSO and 5 mM MgCl2 in the presence of 8 mM ATP for indicated times (Fig. 5a) at 37°C. The reaction was stopped by adding 0.2 mg ml−1 protease K, 50 mM EDTA and 3% SDS (final concentrations) by incubating for 20 min, and then the reaction products were loaded on 1% native agarose gel. DNA was stained with EtBr, visualized by FluorChem Q (ProteinSimple) and quantified by ImageJ software.

ATPase assay. The indicated amount of purified MCMC8-9 was incubated with reaction buffer containing 25 mM HEPES-NaOH (pH 7.5), 50 mM sodium acetate, 1.5 mM Mg(OAc)2 and 1 μCi of [γ-32P]ATP (6,000 Ci mmol−1, Perkin-Elmer) at 60°C at 1 h. Two microlitres of each sample (15 μl) was spotted onto a thin-layer chromatography (TLC) plate (Sigma-Aldrich) followed by development in 1 M formic acid and 0.5 M LiCl, and ATP hydrolysis was visualized by autoradiography using X-ray film (Kodak).

Cell survival assay. U2OS cell lines, which stably expressed WT or mutants of the WA or WB motif of MCM9, and various prostate cancer cells were used for the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The indicated amount of cisplatin (Sigma-Aldrich) was incubated with cells for 24 h, and cell viability was measured by MTT assay, following the manufacturer’s protocol (Promega, CellTiter 96 Non-Radioactive Cell Proliferation Assay (MTT)). To deplete endogenous MCM9 from each cell line, siMCM8 or siMCM9 were transfected into those cell lines 24 h before cisplatin treatment. For the clonogenic assay, U2OS cell lines having WT or mutants of MCM8 or MCM9 were transfected with the indicated siRNA and
plated on a six-well plate after 24 h. The indicated concentration of cisplatin was added for 4 h, beginning at 42 h after siRNA transfection. At day 5 after DNA damage, the colonies were stained with crystal violet. NCI-H292 cells were plated and transfected with the indicated plasmid using Lipofectamine2000 (Invitrogen). After transfection, 10,000 cells of each transfectant were plated into one well of a six-well plate, and incubation with 2 µM of cisplatin was started at 36 h after transfection. Cisplatin was washed out with fresh medium after 24 h. The colonies were stained with crystal violet at 12 days after DNA damage and quantified using Gene Tools software (Syngene).

References

1. Ciccia, A. & Elledge, S. J. The DNA damage response: making it safe to play with knives. Mol. Cell 40, 179–204 (2010).
2. Aparicio, T., Baer, R. & Gautier, J. DNA double-strand break repair pathway choice and cancer. DNA Repair (Amst) 19, 169–175 (2014).
3. Sartori, A. A. et al. Human CtIP promotes DNA end resection. Nature 450, 509–514 (2007).
4. Williams, R. S. et al. Nbs1 flexibly tethers Ctp1 and Mre11-Rad50 to coordinate DNA double-strand break processing and repair. Cell 139, 87–99 (2009).
5. Mimitou, E. P. & Symington, S. L. Nucleases and helicases take center stage in homologous recombination. Trends Biochem. Sci. 34, 264–272 (2009).
6. Imamura, O. & Campbell, J. L. The human Bloom syndrome gene suppresses the DNA replication and repair defects of yeast MCM2 mutants. Proc. Natl Acad. Sci. USA 100, 8193–8198 (2003).
7. San Filippo, J., Sung, P. & Klein, H. Mechanism of eukaryotic homologous recombination. Annu. Rev. Biochem. 77, 229–257 (2008).
8. Shibata, A. et al. DNA double-strand break repair pathway choice is directed by distinct MRE11 nuclease activities. Mol. Cell 53, 7–18 (2014).
9. Lutzmann, M. et al. DNA damage sensing by the DNA replication and repair defects of yeast DNA2 mutants. DNA Repair (Amst) 10, 2761–2772 (2011).
10. Blanton, H. L. Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. Mol. Cell. Biol. 24, 1393–1408 (2004).
11. Long, D. T., Raschle, M., Joukov, V. & Walter, J. C. Mechanism of DNA double-strand break repair by the MRE11/RAD50/XRS2 complex. Cell 120, 315–328 (2005).
12. Kim, H. & D’Andrea, A. D. Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. Genes Dev. 26, 1393–1408 (2012).
13. Mimitou, E. P. & Symington, S. L. Nucleases and helicases take center stage in homologous recombination. Trends Biochem. Sci. 34, 264–272 (2009).
14. Lutzmann, M. et al. DNA MCM8-9 complex promote RAD51 recruitment at DNA double-strand break repair pathway choice. Nature 511, 122–125 (2014).
15. Blanton, H. L. et al. REC, Drosophila MCM8, drives formation of meiotic RPA-ssDNA complexes. Mol. Cell 4, 426–437 (2004).
16. Pierce, A. J., Hu, P., Han, M., Ellis, N. & Jasin, M. Ku DNA end-binding protein modulates homologous repair of double-strand breaks in mammalian cells. Mol. Cell. 15, 3237–3242 (2004).
17. Pierce, A. J., Johnson, R. D., Thompson, L. H. & Jasin, M. XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. Genes Dev. 13, 2633–2638 (1999).

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Author contributions

K.Y.L. and A.D. designed the study, while K.Y.L. executed most of the experiments and the ER-α2UOS plasmids for HR assay and the ER-α2UOS cells, respectively. We thank Drs Maria Jasin and Tanya T. Paull for supplying the plasmids for HR assay and the ER-α2UOS cells, respectively. Also, we thank members of the Dutta laboratory for their helpful discussions. This work was supported by R01 CA60499 and CA166054 (to A.D.).

Additional information

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