**Supplementary Information**

XRCC1 counteracts PARP poisons, Olaparib and Talazoparib, and a clinical alkylating agent, Temozolomide, by promoting the removal of trapped PARP1 from broken DNA

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Supplementary Figure Legends

**Fig. S1 Mechanism of base alkylation induced by MMS, Temozolomide, Carmustine, and Lomustine**

MMS, the model alkylator, predominantly methylates DNA at N\(^7\) of guanine, Temozolomide methylates at N\(^7\) and O\(^6\), and the nitrosoureas, Carmustine and Lomustine, chloroethylate at O\(^6\) of guanine. N\(^7\) and O\(^6\) lesions are repaired by BER, and the latter lesion is also removed by O\(^6\)-methylguanine DNA methyltransferase.

**Fig. S2 A current model for BER and highlights of our manuscript**

The step-wise processes of BER are illustrated. BER usually generates SSBs 5’ to damaged nucleotides irrespective of the type of lesions (c). PARP1 and XRCC1 promote BER at the SSB repair steps (d to h). The PARylation of chromatin proteins recruits ALC1 and XRCC1 (e to f), and it provides docking sites of BER effectors, including Aprataxin, LIG3, PNKP, and POLβ (g). The autoPARylation (e) is believed to play an important role in releasing PARP1 from unrepaired SSBs (f). The current manuscript revealed that XRCC1, but not ALC1 or POLβ, is required for removing PARP1 from unrepaired SSBs (e to f). We also uncovered the modest sensitivity of PARP1\(^{-/-}\)/XRCC1\(^{-/-}\) cells to alkylating agents, indicating that PARP1 and XRCC1 are dispensable for functioning BER effectors.

**Fig. S3 Clinical PARP poisons block BER to a much greater extent than the loss of PARP1 and catalytic PARP inhibitors**

PARP1 plays a role as a damage sensor and facilitates SSB repair (a, b). In PARP1\(^{-/-}\) cells, BER effectors still efficiently seal SSBs (c, d). We here defined the PARP catalytic inhibitor as inhibitors whose cytotoxic effect is similar between wild-type and PARP1\(^{-/-}\) cells, such as Veliparib (e, f). Clinical PARP poisons, Olaparib and Talazoparib, are much more toxic to wild-type cells than PARP1\(^{-/-}\) cells because poisons inhibit the dissociation of PARP1 from SSBs.
thereby interfering with the access of BER factors to SSBs (g, h). Molecular mechanisms underlying the different effects of Veliparib, Olaparib, and Talazoparib on PARP1 remain unclear.

Fig. S4. Disruption of the \textit{PARP1} gene in human TK6 cells

(a) Schematic of part of the \textit{hPARP1} locus. The knockout constructs are shown below the locus. The filled boxes represent exons. The thick lines show the genomic region amplified for targeting-vector arms. Indicated gRNA sequence was inserted into the BbsI site of pX330 (Cat# 42230, Addgene, US). pX330 expresses gRNA under the control of the U6 promoter and Cas9 under the chicken \(\beta\)-actin promoter. pX330-gRNA and indicated two targeting vectors were transfected into TK6 cells using the Neon transfection system (Thermo Fisher Scientific, PA). At 48 h after the transfection, puromycin and neomycin were added to select cells carrying maker genes. (b) \textit{Wild-type} (\(+/+)\) and \textit{PARP1}\(^{-/-}\) TK6 cells were subjected to western blot using an \(\alpha\)-PARP1-specific antibody. The blot was probed with an anti-histone H3 antibody as a loading control.

Fig. S5. Disruption of the \textit{PARP1} gene in human MCF-7 cells

(a) Schematic of part of the \textit{hPARP1} locus. The filled boxes represent exons. Indicated gRNA sequence was inserted into the BbsI site of pX459 (Cat# 48139, Addgene, US). pX459 expresses gRNA under the control of the U6 promoter and Cas9 under the chicken \(\beta\)-actin promoter. pX459-gRNA was transfected into MCF-7 cells, which were seeded on 6 cm dish containing \(\sim\)60% confluency, with Fugene HD (Promega, US) according to the manufacture protocol. At 24 h after the transfection, puromycin was added to a final concentration of 2 \(\mu\)g/ml. The MCF-7 cells were incubated for 48 h with the puromycin-containing medium and further cultured for approximately two weeks to isolate the clones. (b) \textit{Wild-type} (\(+/+)\) and \textit{PARP1}\(^{-/-}\) MCF-7 cells were subjected to western blot using an \(\alpha\)-PARP1-specific antibody. The blot
was probed with an anti-histone H3 antibody as a loading control. The gene-disruption events were confirmed by western blotting analysis.

Fig. S6 Expression of XRCC1 cDNA rescues the hypersensitivity of XRCCI<sup>−/−</sup> MCF-7 cells to TMZ, Olaparib, and Talazoparib.

(a-c) Indicated MCF-7 cells were examined for sensitivity to TMZ (a), Olaparib (b), or Talazoparib (c), as described in Figures 2 and 6.

Fig. S7 XRCCI<sup>−/−</sup> MCF-7 cells are hypersensitive to Carmustine and Lomustine

The indicated MCF-7 cells were assessed for sensitivity to clinically relevant alkylating agents, Carmustine and Lomustine. Cells were treated with the indicated alkylating agent for 24 h and then cultured in a drug-free medium for 14 days, and the number of colonies was counted. The drug dose is displayed on the x-axis on a linear scale, while the percentage of cell survival is displayed on the y-axis on a logarithmic scale. Error bars represent the standard deviation from three independent experiments.

Fig. S8 XRCCI<sup>−/−</sup> cells, but not PARP<sup>−/−</sup>/XRCCI<sup>−/−</sup> cells, show delayed BER kinetics

(a) The alkaline-comet assay to measure the number of unrepaired SSBs after exposure of the indicated TK6 cells to TMZ (0, 150, or 450 µM) for 1 h. (b) The alkaline-comet assay to measure the repair kinetics of SSBs. The indicated TK6 cells were exposed to H<sub>2</sub>O<sub>2</sub> (80 µM) on ice for 30 min and then were released into a drug-free, pre-warmed culture medium for the indicated times. (c) Cells were exposed to H<sub>2</sub>O<sub>2</sub> (80 µM) on ice for 30 min, then released into a pre-warmed culture medium containing Olaparib (1 µM) for the indicated times. Comet-tail moments were measured from at least 50 nuclei in each test. Each experiment was repeated three times.
Fig. S9 The role of XRCC1 in the release of PARP from DNA lesion

Western blot of chromatin-bound fractions prepared from human MCF-7 clones. The indicated cells were treated with TMZ (1 mM) for indicated durations. Blots were probed with the indicated antibodies. Histone H3 was probed as a loading control.

Fig. S10 Slow migrating signal around 250 kDa detected by anti-poly(ADP-ribose)-specific antibody is exhibited in PARP1−/− cells

Auto-PARylation levels in the indicated cells without TMZ treatment were assessed as in Figure 5 a.

Fig. S11 XRCC1−/− cells are over two ordered magnitudes higher sensitive to Olaparib than wild-type, ALC1−/−, and POLβ−/− cells

(a, b) The TK6 cells carrying the indicated genotypes were assessed for sensitivity to Olaparib. Cells were cultured in methylcellulose medium containing Olaparib for 14 days. The drug dose is shown on the x-axis on a linear scale (a) or a logarithmic scale (b), and the percentage of colony survival is displayed on the y-axis on a logarithmic scale. Error bars represent the standard deviation from three independent experiments.
(a) Base damage

DNA glycosylase

Abasic site

AP-endonuclease

SSB

(d)

(h)

(PARP1

Chromatin proteins

PAR

XRCC1

BER effectors

Hirota et al. Fig. S2
(a) *Wild-type*

(b) +PARP poison

(c) PARP1-deficient

(d) +PARP1 catalytic inhibitor

(e) +PARP catalytic inhibitor

(f) +PARP catalytic inhibitor

(g) +PARP catalytic inhibitor

(h) +PARP catalytic inhibitor

**Key:**
- PARP1
- Chromatin proteins
- PAR
- BER effectors
(a) PARP1 disruption
gRNA
GAAGTACGTGCAAGGGGTGTATGG

(b) 

| +/+ | -/- |
|-----|-----|
| α-PARP1 |       |
| α-histone H3 |   |
(a) PARP1 disruption

![Diagram](attachment:Image.png)

- gRNA: GAAGTACGTGCAAGGGGTGTATGG
- ATG

(b) 

+/- +/+

α-PARP1

α-histone H3

Fig. S5
**Fig. S7**

Viability (%)

0 1 10 50 100 (μM)

MCF-7 cells
- **Wild-type**
- **PARP1⁻/⁻**
- **XRCC1⁻/⁻**
- **PARP1⁻/⁻/XRCC1⁻/⁻**
(a) Untreated 150 μM TMZ 450 μM TMZ

Tail Moment

** Wild-type
XRCC1−/−
PARP1−/−
PARP1−/−/XRCC1−/−

(b) H₂O₂ ➔ Drug free

Tail Moment

Wild-type
XRCC1−/−
PARP1−/−
PARP1−/−/XRCC1−/−

(c) H₂O₂ ➔ Olaparib

Tail Moment

Wild-type
XRCC1−/−
PARP1−/−
PARP1−/−/XRCC1−/−

(min)
| MCF-7 cells | Wild-type | XRCC1<sup>−/−</sup> |
|-------------|-----------|------------------|
|             | 0 | 1 | 2 | 0 | 1 | 2 |
| α-PARP1     | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| α-histone H3| ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |
Fig. S10

Poly(ADP-ribose) levels in Wild-type and PARP1−/− cells.
## Supplementary Table S1. List of mutant genotypes used in this study

| Genotype     | Name of Cell lines and species | Makers genes | Source                                      |
|--------------|---------------------------------|--------------|---------------------------------------------|
| Wild-type    | Human TK6                        |              | https://cellbank.nibiohn.go.jp/~cellbank/cgi-bin/search_res_det.cgi?ID=6560 |
| PARP1<sup>−/−</sup> | Human TK6                        | puro<sup>R</sup>, neo<sup>R</sup> | This study                                  |
| XRCC1<sup>−/−</sup> | Human TK6                        | his<sup>R</sup>, bsr<sup>R</sup>  | (Saha et al. 2020)                          |
| ALC1<sup>−/−</sup> | Human TK6                        | puro<sup>R</sup>, neo<sup>R</sup> | (Tsuda et al. 2017a)                        |
| POLβ<sup>−/−</sup> | Human TK6                        | puro<sup>R</sup>, neo<sup>R</sup> | (Saha et al. 2020)                          |
| XRCC1<sup>−/−</sup>/PARP1<sup>−/−</sup> | Human TK6                        | puro<sup>R</sup>, neo<sup>R</sup>, his<sup>R</sup>, bsr<sup>R</sup> | This study                                  |
| ALC1<sup>−/−</sup>/PARP1<sup>−/−</sup> | Human TK6                        | puro<sup>R</sup>, neo<sup>R</sup>, his<sup>R</sup>, bsr<sup>R</sup> | This study                                  |
| POLβ<sup>−/−</sup>/PARP1<sup>−/−</sup> | Human TK6                        | puro<sup>R</sup>, neo<sup>R</sup>, his<sup>R</sup>, bsr<sup>R</sup> | This study                                  |
| Wild-type    | Human MCF-7                      |              | https://cellbank.nibiohn.go.jp/~cellbank/cgi-bin/search_res_det.cgi?ID=1543 |
| PARP1<sup>−/−</sup> | Human MCF-7                      | puro<sup>R</sup>, neo<sup>R</sup> | This study                                  |
| XRCC1<sup>−/−</sup> | Human MCF-7                      | his<sup>R</sup>, bsr<sup>R</sup>  | This study                                  |
| XRCC1<sup>−/−</sup>/PARP1<sup>−/−</sup> | Human MCF-7                      | puro<sup>R</sup>, neo<sup>R</sup>, his<sup>R</sup>, bsr<sup>R</sup> | This study                                  |