Supplementary Data

Figure Legends

Supplemental Figure S1. Expression of FLAG-BRCA2 proteins used for the homologous recombination assay.

293T cells were transfected with indicated FLAG-tagged BRCA2 constructs using Lipofectamine Plus according to the manufacturer’s protocol. After 48 hours, cells were lysed in NETN (0.5% NP-40; 20mM Tris pH 8.0; 100mM NaCl; 1mM EDTA) buffer and western blotting was done using anti FLAG (Sigma F1804) or anti β-actin (Sigma A1978).

Supplemental Figure S2. BRCA2 restoration is critical for acquired drug resistance of cisplatin-selected cells. (Full-length blots are presented in Supplemental Fig. S5E)

A. BRCA2 western blotting of PEO1, PEO4, C4-2, C4-5 and C4-11 cells treated with indicated siRNA. BRCA2 siRNA #2 was used in this experiment.

B. Cisplatin sensitivity assessed by crystal violet assay. BRCA2 siRNA (#2)-treated C4-2 and C4-5 cells were cisplatin-sensitive compared to control siRNA-treated cells (p<0.05, LD_{50} data were compared by unpaired t test). BRCA2 siRNA (#2)-treated PEO4 cells tended to be cisplatin-sensitive compared to control siRNA-treated cells, but the difference is not statistically significant (p=0.073). BRCA2 siRNA #2 had no effect on cisplatin sensitivity of PEO1 and C4-11 cells. (mean ± SEM, n=3)

C. AG14361 sensitivity assessed by crystal violet assay. BRCA2 siRNA (#2)-treated PEO4, C4-2 and C4-5 cells were AG14361-sensitive compared to control siRNA-treated cells (p<0.05, LD_{50} data were compared by unpaired t test). BRCA2 siRNA #2 did not
sensitize PEO1 or C4-11 cells to AG14361. (mean ± SEM, n=3)

Supplemental Figure S3. Restoration of functional BRCA2 by secondary BRCA2 mutation in PARP inhibitor-selected PEO1 clones. (Full-length blots are presented in Supplemental Fig S5F-G)

A. BRCA2 western blotting of the 3 clones (AG4-8, AG4-11 and AG4-12) of PEO1 generated by selecting the cells in the presence of AG14361. A double asterisk indicates a band presumed to be non-specific.

B. Genomic DNA sequence of BRCA2 in AG4-11. In addition to the original mutation (5193C>G), a secondary mutation (5192A>T) was observed. The same mutation was observed in AG4-8 and AG4-12 (data not shown). The mutation was confirmed in cDNAs (data not shown).

C. Ionizing radiation (IR)-induced RAD51 foci formation is restored in the BRCA2-restored PEO1 clones. Quantification of the cells with at least five RAD51 foci before (-, white bars) and 12 hours after IR (+, grey bars) is shown. Asterisks (*) indicate significant difference with irradiated parental PEO1 cells (p<0.05, unpaired t test). (mean ± SEM, n=3)

D. Cisplatin/PARP inhibitor sensitivity assay of AG14361-selected PEO1 clones. The 3 BRCA2-restored clones were resistant to both cisplatin and AG14361 compared to the parental PEO1 cells. The cells were treated with cisplatin or AG14361 at the indicated concentrations for 8 days, and survival fraction was measured by crystal violet assay. All of the AG14361-selected PEO1 clones and PEO4 are cisplatin/AG14361 resistant compared to parental PEO1 (p<0.05, LD50 data were compared by unpaired t test).
(mean ± SEM, n=3)

E. BRCA2 western blotting of AG4-8, AG4-11 and AG4-12 cells treated with indicated siRNA. BRCA2 siRNA #1 was used in this experiment.

F. Cisplatin/PARP inhibitor sensitivity assay. Depletion of BRCA2 by siRNA sensitized AG4-8, AG4-11 and AG4-12 to cisplatin and AG14361. BRCA2 siRNA (#1)-treated AG4-8, AG4-11 and AG4-12 cells were cisplatin/AG14361-sensitive compared to control siRNA-treated cells (p<0.05, LD50 data were compared by unpaired t test). (mean ± SEM, n=3).

Supplemental Figure S4. BRCA2 restoration is critical for acquired drug resistance of AG14361-selected cells. (Full-length blots are presented in Supplemental Fig. S5H)

A. BRCA2 western blotting of PEO1, AG4-8, AG4-11, and AG4-12 cells treated with indicated siRNA. BRCA2 siRNA #2 was used in this experiment.

B. Cisplatin/PARP inhibitor sensitivity assessed by crystal violet assay. BRCA2 siRNA (#2)-treated AG4-8, AG4-11 and AG4-12 cells were cisplatin/AG14361-sensitive compared to control siRNA-treated cells (p<0.05, LD50 data were compared by unpaired t test). (mean ± SEM, n=3).

Supplemental Figure S5. Non-cropped pictures of blots.

(A-B) Non-cropped pictures of blots presented in Figure 1A.

(C) Non-cropped pictures of blots presented in Figure 2A.

(D) Non-cropped pictures of blots presented in Figure 4A.

(E) Non-cropped pictures of blots presented in Supplemental Figure S1A.
(F) Non-cropped pictures of blots presented in Supplemental Figure S2A.

(G) Non-cropped pictures of blots presented in Supplemental Figure S2E.

(H) Non-cropped pictures of blots presented in Supplemental Figure S3A.
[A] Ag4-11, BRCA2 5192A>T; 5193C>G (Y1655L) 
exon 11

[B] Cells with five or more RAD51 foci (%)

[C] Survival fraction (%)

[D] Survival fraction (%)

[E] Survival fraction (%)

[F] Survival fraction (%)

Sakai, et al.- Supplemental Figure S3