RAD51C-deficient cancer cells are highly sensitive to the poly (ADP-ribose) polymerase inhibitor, olaparib.

Ahrum Min\textsuperscript{a}, Seock-Ah Im\textsuperscript{a,b,*}, Young-Kwang Yoon\textsuperscript{a}, Sang-Hyun Song\textsuperscript{a}, Hyun-Jin Nam\textsuperscript{a}, Hyung-Seok Hur\textsuperscript{a}, Hwang-Phill Kim\textsuperscript{a}, Kyung-Hun Lee\textsuperscript{a,b}, Sae-Won Han\textsuperscript{a,b}, Do-Youn Oh\textsuperscript{a,b}, Tae-You Kim\textsuperscript{a,b,c}, Mark J O’Connor\textsuperscript{e}, Woo-Ho Kim\textsuperscript{a,d} and Yung-Jue Bang\textsuperscript{a,b,*}

\textsuperscript{a}Cancer Research Institute, Seoul National University
\textsuperscript{b}Department of Internal Medicine, Seoul National University College of Medicine
\textsuperscript{c}Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology
\textsuperscript{d}Department of Pathology, Seoul National University College of Medicine
\textsuperscript{e}AstraZeneca UK Ltd., Macclesfield, Cheshire, United Kingdom

*Requests for reprints: Co-Corresponding Authors

Yung-Jue Bang, M.D., Ph.D., and Seock-Ah Im, M.D., Ph.D.

Department of Internal Medicine
Seoul National University College of Medicine
101 Daehak-ro, Jongno-gu, Seoul, 110-744, Korea
Tel: +82-2-2072-2390, and 82-2-2072-0850
Fax: 82-2-762-9662
E mail: bangyj@snu.ac.kr and moisa@snu.ac.kr
Supplementary Figure Legends

Figure S1

Olaparib has variable levels of anti-tumor activity in human cancer cell lines. A. The anti-tumor activity of olaparib was measured with a CFA. Cells were seeded and cultured with increasing doses of olaparib for 14 d. The cell colonies were stained and counted. IC\textsubscript{50} values were calculated and are represented in the table. B. The growth inhibitory activity of olaparib was evaluated by a CFA. Cells were treated with increasing doses of olaparib for 14 d. The percentage of surviving cells was calculated by counting the number of colonies and IC\textsubscript{50} was calculated using SigmaPlot (SNU-601, 0.037 ± 0.001 µmol/L; SNU-668, 3.80 ± 0.670 µmol/L; BT-549, 0.054 ± 0.003 µmol/L; and MCF-7, 6.872 ± 0.325 µmol/L) and presented as a graph with standard error bars (n=4).

Figure S2

Olaparib sensitive cell lines have different patterns of gene expression compared to insensitive cell lines. A. The expression levels of RAD51 paralogs were analyzed by western blotting. Cells were harvested 24 h after plating and immunoblotting was performed with the indicated antibodies. \textalpha-tubulin was used as a loading control. B. The expression levels of DNA repair molecules were determined by RT-PCR. Total RNA was isolated from SNU-601 and SNU-668 cells, and the indicated genes were amplified with specific primers.

Figure S3

PTEN expression does not affect olaparib sensitivity. A. pCMV-Tag2B or pCMV-Tag2B/PTEN constructs were transiently expressed in BT-549 cells. The cells were then exposed to increasing doses of olaparib for 10 d. Cell survival was measured in the presence of olaparib with a CFA and expressed as a percentage. These experiments were repeated twice. Bars, ± SD. B. PTEN over-expression was confirmed by western blot analysis.
Figure S4

**RAD51C expression affects olaparib sensitivity in human cancer cell lines.** *A.* The effect of increasing doses of olaparib was determined using a CFA. The SNU-668 cells were transfected control or RAD51C-specific siRNA and non-transfected SNU-601 cells were exposed to increasing doses of olaparib. After 14 d, the colonies were stained with 0.1% Coomassie Blue solution and counted. *B.* The pcDNA3 or pcDNA3-RAD51C plasmids were used to transflect SNU-601 cells, and stable clones were selected with G418 treatment. After 14 d of olaparib treatment, the growth inhibitory effect of olaparib on the stable clones was measured by a CFA.

Figure S5

**RAD51C over-expression decreases sensitivity to olaparib in the BT-549 cell line.** *A.* The BT-549 cells were transfected with the pcDNA3 or pcDNA3-RAD51C plasmid and treated with increasing doses of olaparib for 5 d after transfection. Cell survival was measured with an MTT assay and is expressed as a percentage. *Bars,* ± SD. *B.* RAD51C over-expression was observed by western blot. These experiments were repeated three times.

Figure S6

**Olaparib induces G2/M cell cycle arrest and apoptosis in sensitive cell lines.** BT-549 and MCF-7 cells were treated with the indicated concentrations of olaparib for 4 d. The percentages of cells in the G2/M or sub-G1 phase were determined by FACS analysis. *a,* p = 0.043; *b,* p = 0.0009. *Bars,* ± SE.

Figure S7

**RAD51C depletion increases the accumulation of DNA damage.** *A.* Cells were treated with 1 μmol/L olaparib for 48 h and comet assay was conducted. Quantification of the comet tail moments in cells is presented in the bar graph (*Left*). Representative images of comet tails are shown in the panels (*right*). *a,* p = 0.0023; *b,* p = 0.005. *B.* The cells were exposed to 10 Gy of radiation and comet tail moments were calculated. The assay was repeated three times and result is shown in the bar graph.
The columns represent the mean of three independent experiments and are shown with error bars (± SE). a, p = 0.0046; b, p = 0.0065 C. The siRNA-mediated reduction of RAD51C expression was confirmed by western blot.

**Figure S8**

**RAD51C expression decreases in tumor tissues.** A. The level of RAD51C protein expression in 11 normal and tumor tissue samples was determined by western blot. α-tubulin was used as a loading control. B. Representative examples of RAD51C expression detected by IHC staining in gastric tumor tissues are shown in the panels. The scale bars represent 50 μm.

**Figure S9**

**RAD51C expression is down-regulated in gastric tumor tissues via DNA methylation.** A. The SNU-601 and SNU-668 cells were incubated with DMSO or 5-aza-dc for 3 d. The total RNA was then extracted. Quantitative real-time PCR was used to measure the induction of RAD51C expression in the cells treated with 5-aza-dc. Bars, ± SE. B. The methylation status of the RAD51C gene in human tumor and normal tissue samples was analyzed by genomic sequencing following bisulfite modification. Thirteen pairs of normal and tumor tissues were randomly selected and three clones per sample were chosen for analysis.