Supplemental Information

SI methods

Cell culture

The human breast carcinoma cell line MCF7 (HTB-22) was obtained from ATCC (Manassas, VA) and maintained in DMEM (GIBCO, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Atlanta Biologicals). MCF7\textsuperscript{DRGFP}, derived from the well-established MCF7 cell line and carrying a single copy of a chromosomally integrated HR substrate, was used to measure HR-mediated DNA repair. MCF7 cells stably expressing BRCA1 shRNA were obtained courtesy of Dr. Simon Powell (Memorial Sloan Kettering, NY). MCF7\textsuperscript{DRGFP} and MCF7 BRCA1 shRNA cells were maintained in DMEM supplemented with 10% FBS and 2μg/mL puromycin (Sigma). The genetic background, including expression and function of key proteins as well as the growth characteristics and their response to genotoxic agents, was tested most recently in December 2011 using western blot analysis, immunohistochemistry, and colony formation assays. The PARP inhibitor ABT-888 (Enzo Life Sciences) was utilized in our study. All transfections were performed using FuGene6 according to the manufacturer’s recommendations (Roche).

Immunofluorescence staining

BRCA1 location was assayed as described previously (1-3). Breast cancer cell lines were cultured and seeded on sterile cover slips and subjected to various treatments as indicated. Cells were fixed at the indicated time points, blocked and incubated with primary antibody [1:100 dilution, anti BRCA1 (ab-1), EMD chemicals Inc, catalog # OP-92, or 1:500 dilution, anti-YFP, Santa Cruz Biotechnology, catalog # sc-32897]. To assay HR-mediated repair, breast cancer cell lines were cultured and seeded on sterile cover slips, exposed to 4 Gy irradiation using an X-ray
irradiator (Kimtron Inc., Woodbury, CT). Cells were fixed at the indicated time points and immunohistochemistry was performed to detect Rad51 foci, a well-established marker for HR-mediated repair, as previously described (4). To assay DNA DSBs breast cancer cell lines were cultured and seeded on sterile cover slips, exposed to 4Gy IR. 24 hours later, they were treated with ABT-888 (10μM) or vehicle control. Cells were fixed at the indicated time points and γ-H2AX immunohistochemistry was performed as previously described (4, 5). Briefly, cells were rinsed in phosphate buffered saline (PBS) and incubated for 5 minutes at 4°C in ice-cold cytoskeleton buffer (10mM Hepes/KOH, pH 7.4, 300mM sucrose, 100mM NaCl, 3mM MgCl₂) supplemented with 1mM PMSF, 0.5mM sodium vandate and proteasome inhibitor (Sigma, 1:100 dilution) followed by fixation in 70% ethanol for 15 minutes. The cells were blocked and incubated with primary antibodies [1:500 dilution, Rad 51 (H-92), Santa Cruz Biotechnology, catalog # sc-8349, or phospho H2AX Ser139, Millipore, catalog # MI-07-164]. The secondary antibody was anti-rabbit Alexa Fluor 594-conjugated or anti-mouse Alexa Fluor 488-conjugated antibody (1:2000 dilution; Invitrogen). DAPI (Invitrogen, catalog # D21490) was used for nuclear staining. The cover slips were subsequently mounted onto slides with mounting media (Aqua poly mount, Polysciences, Inc. catalog # 18606) and analyzed via fluorescence microscopy (Carl Zeiss, Thornwood, NY). Positive and negative controls were included on all experiments. A total of 500 cells were assessed according to the standard procedure (4).

**Cell cycle analysis**

Cell cycle distribution was measured as previously described (6). 2x10⁵ cells were seeded in 100 mm² dishes and treated with mock or 4Gy irradiation. 8 hours post-radiation treatment, cells were transfected with ISce-1 or empty vector. Cells were collected and fixed at different time points, treated with RNAse (Sigma, catalog # R-4875), stained with propidium iodide (PI),
and read on FACS Calibur using CellQuest. Data was analyzed using ModFit LT by Verity Software Inc.

**Clonogenic survival assay**

Cell survival was evaluated by the colony formation assay in the breast cell lines following radiation treatment and various doses of ABT-888 (1μM-10μM) as previously described (7). Briefly, cells were seeded and irradiated with 4Gy. 24 hours later they were subjected to the indicated doses of ABT 888 (or vehicle). The plates were left undisturbed and three weeks following treatment, colonies were fixed with 70% ethanol, stained 1% methylene blue and number of positive colonies were counted (>50 cells). Survival fraction was calculated as follows: (number of colonies for treated cells/number of cells plated)/(number of colonies for corresponding control/number of cells plated). Experiments were performed in triplicate.

**Tumor growth delay**

Tumor cells (10^7) were injected subcutaneously into the left and right hind limbs of 6 weeks old female athymic Foxn1 (nu/nu) mice (Harlan Sprague Dawley, Inc). Once tumors reached 250 mm^3, mice were randomized into four treatment groups (n=3): control, IR (3Gy, single dose to hind limb), ABT-888 (25 mg/kg, daily for 5 days beginning 24 hours after IR), IR+ABT-888. Tumor size was measured on alternate days for 21 days, at which point tumors were harvested and fixed in 10% formalin. Tumor volume was calculated using the following formula: \( \frac{1}{2} \times \text{length} \times \text{width}^2 \). Paraffin sections were stained with hematoxylin and eosin. Immunohistochemical detection of \( \gamma \)-H2AX and BRCA1 were performed as described previously (1, 8). Briefly, tissues were incubated with primary antibodies [1:50 dilution, BRCA1 (Abcam, catalog # OP-92) and \( \gamma \)-H2AX Ser139 (Millipore, catalog # MI-07-164)]. Secondary antibodies include Alexa Fluor 488–conjugated antibody or Alexa Fluor 594–conjugated
antibody (1:500 dilution, Invitrogen). DAPI (Invitrogen, catalog # D21490) was used for nuclear staining. All animal procedures were approved by the Vanderbilt University Institutional Animal Care and Use Committee (protocol M/08/154).
Reference


