SUPPLEMENTARY INFORMATION

EXTENDED MATERIALS AND METHODS

Cell culture

900677A (primary) and 900677AT (large T antigen immortalized) fibroblasts with a genetic deficiency for *XRCC2*, and derivative lines, and HEK293T, U2OS-DR, and GM0637 cells, were grown in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were irradiated as described previously.[1] Stock solutions of cycloheximide (10 mg/ml in H_2O; Sigma), MG132 (10 mM in DMSO; Calbiochem), olaparib (10 mM in DMSO; Sigma), MMC (3 mM in 50% ethanol; Sigma), and HU (500 mM in medium; Sigma) were kept at -20^{o}C.

Cloning and mutagenesis

Human XRCC2 cDNA (SC116732) was purchased from Open Biosystems. The R215X mutant of XRCC2 was generated using the QuikChange II Site-Directed Mutagenesis kit (Stratagene) according to the manufacturer's instructions.

Transfection and viral transduction

900677AT cells were reconstituted with XRCC2 or a neomycin phosphotransferase containing control S91 gammaretroviral vector for selection with G418/geneticin, as described previously.[2 3] Alternatively, for experiments involving the R215X mutant of XRCC2, cells were retrovirally transduced (pOZ) with the mutant or XRCC2-WT along with a N-terminal Flag-HA tag and selected by IL-2 beads as described previously.[4]

Immunofluorescence microscopy

Cells were grown on coverslips coated with poly-D-lysine, fixed with 2% paraformaldehyde for 20 min and permeabilized with 0.2% Triton X-100 for 3 min. Following washes with PBS, slides were incubated with primary and secondary antibodies, washed, and mounted with a coverglass, as described previously.[5]

Microscopy, collection of images, counting of three replicates per sample, and the generation of figures were as described previously.[5]

Immunoprecipitation

Cells were lysed in NETN 420 buffer supplemented with protease inhibitors and PMSF as described previously.[1] Immunoprecipitations were performed with anti-Flag M2 Affinity Gel (Sigma) or specified antibodies as described previously.[1]

Antibodies

The following primary antibodies were utilized: RAD51D (Novus, NB100-166, rabbit), XRCC2 (Santa Cruz, sc-5895, goat and sc-365854, mouse), BRCA1 (Millipore, 07-434, rabbit) and BRCA2 (Calbiochem, OP95, mouse). Anti-RAD51C (mouse), anti-XRCC3 (rabbit), anti-γH2AX (mouse), anti-HA (mouse), anti-β actin (mouse) antibodies were as described elsewhere.[6]

Secondary antibodies for immunofluorescence microscopy included FITC conjugated donkey anti-mouse or anti-rabbit IgG and Rhodamine B-conjugated donkey anti-mouse or anti-rabbit IgG antibodies (Jackson Immunoresearch) as described previously.[5] For

immunoblotting, signals from HRP-linked secondary antibodies (Amersham) were detected by chemiluminescence (Amersham) as previously described.[5]

DNA damage sensitivity assays

For measurements of MMC, olaparib and formaldehyde sensitivity, cells were plated into 96 well plates in triplicate at $2X10^3$ cells/well. The next day, cells were treated with MMC at doses ranging from 0-400 nM, olaparib at doses from 0-4 μM, and formaldehyde at doses from 0-80 μM. Medium was aspirated and fresh medium without any of these DNA damage agents was added after overnight incubation, and cells were incubated an additional 3-4 days. Relative survival was measured using a colorimetric assay as described.[1]

For measurements of sensitivity to IR, 900677AT cells, with or without correction with XRCC2, were plated into 6 cm dishes in triplicate after irradiating with doses ranging from 0-8 Gy. Cells were incubated for 10 days and the number of colonies/dish was counted as described.[6]

Chromosome breakage analysis

For assays of chromosome breakage in primary cells, cultures in T75 flasks in DMEM medium containing 20% fetal bovine serum, as well as penicillin-streptomycin and L-glutamine, were exposed to 0, 0.01 or 0.1 μg/ml diepoxybutane (DEB) for 48 hr. Cells were accumulated in mitosis by treatment with 0.2 mg/ml colcemid for the last 7-8 hr. Metaphase spreads were prepared, G banded, and analyzed under a brightfield microscope.

For assays of Lg T-immortalized lines, cells were grown in a T25 flask and were treated with 300 nM MMC for 72 hr in the dark, and arrested in mitosis with a final concentration of 200

ng/ml of colcemid (Gibco) for 3 hr. Cells were collected by trypsinization, washed with medium containing serum, and treated with 50 mM KCl for 10 min at 37^{o}C for swelling. Cells were subsequently fixed, dropped onto slides, and stained with Giemsa as described,[7] and visualized using a microscope.

The total number of aberrations per chromosome, including breaks, gaps and radials was counted as described.[8] The percent of cells with one or more radial chromosomes was also calculated. Each count consisted of 3 sets of 30 metaphases each which was utilized for a mean $\pm$ standard deviation.

G2-M accumulation

To measure G2-M accumulation, cells were treated with 0.35 μg/ml of melphalan, fixed, stained with propidium iodide and treated with RNAse A, gated, and analyzed using ModFit software as described.[4]

REFERENCES FOR EXTENDED MATERIALS AND METHODS

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