**Supplementary Methods**

**Acquired *RAD51C* promoter methylation loss causes PARP inhibitor resistance in high grade serous ovarian carcinoma**

Nesic *et al.*

*Reagents*

Niraparib (TesaroBio) was purchased from MedChemExpress, and Rucaparib was provided by Clovis Oncology. Mouse anti-human RAD51C antibody (sc-56214) was purchased from Santa Cruz Biotechnology, goat anti-beta actin (ab8229), Rabbit anti-human RAD51 (ab133534) and mouse anti-human Geminin antibodies (ab104306) from Abcam, Human-specific EpCam-647 (clone VU1D9, #5447) and rabbit anti-human H2AX antibodies (Phospho-Histone H2A.X Ser139;clone 20E3, #9718) from Cell Signaling Technologies, and EpCam-APC antibody (clone EBA-1, #347200) from BD Biosciences. LIVE/DEAD Fixable Aqua Dead Cell Stain Kit, for 405 nm excitation was purchased from Invitrogen (#L34957). The following antibodies were used for immunohistochemistry: p53 (M700101 1:100; Dako), Ki67 (M7240 1:50; Dako), Cytokeratin (Pan-CK; M3515 1:200; Dako), PAX8 (10336–1-AP 1:20000; Proteintech), and WT1 (ab15249; 1:800; Abcam).

*HGSC Patient Derived Xenograft - Patient Details*

PDX #183 was from a 65 year-old patient diagnosed with bulky stage III HGSC, and was confirmed to be HGSC by histological staining (Supplementary Fig. 1). PDX was established following surgical de-bulking. This patient was subsequently treated with paclitaxel and carboplatin chemotherapy and bevacizumab in the first-line. The patient responded to first-line treatment, with a treatment-free interval (TFI) of 11 months. Second-line therapy of paclitaxel and carboplatin chemotherapy resulted in a reduced TFI of 7 months. The TFI was shorter again for third-line therapy of carboplatin and gemcitabine, where it was 4 months. This patient died from the disease 3 years and two months from diagnosis, having never received PARPi. PDX #1240 was derived from ovary tumor material from primary surgery in a 58 year-old woman diagnosed with stage IIIB HGSC. At last follow-up the patient had experienced a para-aortic (PA) node recurrence less than six months following completion of platinum-based chemotherapy, and was prescribed high dose palliative radiotherapy to the PA region combined with radio-sensitizing cisplatin.

*Targeted RAD51C bisulfite amplicon sequencing details*

Bisulfite-converted DNA (bisDNA) samples were not quantitated prior to PCR, but were instead quantitated and normalized prior to sequencing (DNA was 25ng/ul prior to bisulfite conversion). For the inner PCR reaction, 4 µl of bisDNA was added to 2 µl 10x PCR buffer (Qiagen, Cat# 203203), 2 µl of 10 µM primer mix (Supplementary Methods Table 1), 0.4 µl of 10 µM dNTP mix, 11.5 µl of molecular grade H2O and 0.1 µl HotStarTaq DNA Polymerase (Qiagen, Cat# 203203). \

**Supplementary Methods Table 1. Primers for targeted bisulfite sequencing of the *RAD51C* promoter.** Product size excludes Illumina adaptors and barcodes, includes targeted primers. Bp – base pairs.

|  |  |  |  |
| --- | --- | --- | --- |
| **Primers** | **Primer sequence** | **Product size (bp)** | **Annealing Temperature (Ta) Cº** |
| *bisRAD51C* forward primer **(illumina adaptors bold)** | **TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**GAAAATTTAtAAGAtTGCGtAAAGtTGtAAGG | 142 | 60 |
| *bisRAD51C* reverse primer **(illumina adaptors bold)** | **GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CTAACCCCGaAaCAaCCAAACTCC |

Reactions were incubated at 95°C for 15 minutes, followed by 15 cycles of 95°C for 30 seconds, 60°C for 40 seconds, 72°C for 40 seconds. Reactions were then incubated at 72°C for 10 minutes, and stored at 4°C. In the outer PCR, unique combinations of Nextera XT Index Kit v2 primers (Illumina) were added for sample multiplexing. Briefly, 2 µl of inner PCR product was added to 2 µl 10x PCR buffer (Qiagen, Cat# 203203), 0.4 µl of 10 µM dNTP mix, 13.5 µl of molecular grade H2O, 0.1 µl HotStarTaq DNA Polymerase (Qiagen, Cat# 203203) and 1 µl of each Nextera XT Index Kit v2 set B or set C barcoding primer (Illumina, Cat# FC-131-2002 or FC-131-2003). These reactions were then incubated at 95°C for 15 minutes, followed by 20 cycles of 94°C for 30 seconds, 60°C for 40 seconds, 72°C for 40 seconds. Reactions were then incubated at 72°C for 10 minutes, and stored at 4°C. Resulting libraries were cleaned-up using Agencourt AMPure XP (Beckman Coulter, Cat#10136224) beads using a ratio of 0.9:1. The D1000 ScreenTape System (Agilent, Cat#5067- 5582 and 5067- 5583) was used to assess the size profile of NexteraXT libraries according to manufacturer’s protocol. NGS libraries were quantitated using the Qubit dsDNA high sensitivity (HS) assay, normalized to 2 nM and pooled. These pools were then sequenced in the WEHI Genomics Core Laboratory on the Illumina MiSeq using a MiSeq Nano Reagent Kit v2 (300 cycle; Illumina; Cat# MS-102-2002) according to manufacturer’s protocol. Libraries were sequenced to a minimum depth of 5,000. Demultiplexed reads were merged and grouped using AmpliVar genotyping mode (1), then processed using a custom script, available on github (https://github.com/okon/MethAmplicons). Reads with deletions, insertions or substitution variants in the CpG positions were filtered out. Epialleles below 5% frequency were grouped together for individual epiallele plots as they were more likely to contain sequencing or bisulfite conversion errors. Sequence data has been deposited at the European Genome-phenome Archive (EGA), which is hosted by the EBI and the CRG, under accession number EGAS00001005395. Further information about EGA can be found on https://ega-archive.org "The European Genome-phenome Archive of human data consented for biomedical research" (2).

*RNA expression analysis in PDX tumors*

RNA was isolated from snap-frozen tissue using the Direct-zol RNA MiniPrep kit (Zymo Research, Cat# R2050) and quality was assessed using the RNA ScreenTape System (Agilent) according to manufacturer’s protocol. The qRT-PCR was performed in triplicate using 100 ng of RNA and the TaqMan RNA-to-CT 1-Step Kit (Applied Biosystems, Carlsbad, CA) per manufacturer’s instructions. Using GAPDH (4352665, Life Technologies) and RAD51C (Hs04194939\_s, Applied Biosystems, Carlsbad, CA) probe sets, PCR was performed on a CFX384 Real Time System (C10000 Touch Thermal Cycler, BioRad, Hercules, CA) using a program consisting of 48°C for 15 min, 95°C for 10 min, then 40 cycles of 95°C for 15 sec and 60°C for 1 min. Data were analyzed using the following equations: ΔCt=Ct(sample)-Ct(endogenous control); and fold-change = 2-ΔCt. Relative *RAD51C* fold-change expression values were normalized to PEO1 cell line control for comparison. RNA sequencing library preparation and data analysis were performed as previously described (3).

*RAD51 foci formation in PDX tumors*

PDX tumors were either harvested (untreated) or irradiated (*in vivo* at 5Gy) and harvested 4 hours post-irradiation at a 400-700 mm3 volume. Tumors were dissociated by mincing followed by a 45 minute incubation at 37°C in digestion media (DMEM/F-12 with GlutaMAX (Gibco), 10 mg/ml Pronase (Sigma-Aldrich) and 1mg/ml DNAse (Sigma-Aldrich)). After digestion, cells were passed through a 100µm cell strainer to create single-cell suspensions. Cells were then stained with LIVE/DEAD Fixable Aqua Dead Cell Stain according to manufacturer’s instructions, and blocked in 2.4G2 supernatant diluted 1:2 in 3% FCS in DPBS, or 7% FCS in DPBS, for a 15 minutes on ice. Cells were stained with human-specific EpCam-647 antibody or EpCam-APC for a minimum dilution of 1:100 or 1:20 antibody respectively. Cells were then fixed with 200-1000µl of 4% Paraformaldehyde for 5 minutes. Cells were sorted for the EpCam-647/APC positive (epithelial cells) and LIVE/DEAD-aqua negative (alive following digestion) population.

Resulting cells were ultra-concentrated in 10 µl of DPBS and plated onto wells of a CellCarrier-96 Ultra Microplates (PerkinElmer, Cat# 6055302). Plates were then centrifuged at 1800g for 10 minutes to allow the cells stick to the surface of the wells. They were then immediately permeabilized using 0.2% Triton-X-100, incubated for 10 minutes at room temperature. Cells were washed (3 times with 150 µl DPBS per well), and blocked in IFF buffer (DPBS with 2% FCS and 1% BSA) for 1 hour (room temperature). Cells were then washed and incubated with anti-Geminin antibody diluted 1:100 in IFF buffer for 1 hour (room temperature). Cells were washed again and incubated overnight at 4°C with either anti-RAD51 or anti-H2AX antibody, diluted 1:250 and 1:200 in IFF buffer respectively. The following day, cells were washed and incubated for 30 minutes at room temperature with a solution of Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 (Invitrogen, Cat# A-11008) and Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 546 (Invitrogen, Cat# A-11030) – both diluted 1:800 in IFF buffer, combined with 1 drop of Hoechst 33342 Ready Flow™ Reagent (Invitrogen, Cat# R37165) per ml of solution. Cells were then washed once in 150 µl of DPBS and left in 150 µl of DPBS until imaging.

*DNA repair foci imaging and analysis*

Cells stained for DNA repair foci on CellCarrier-96 Ultra Microplates (PerkinElmer, Cat# 6055302) were imaged on the Opera Phenix™ High Content Screening System (PerkinElmer). The imaging settings were as follows: optical mode was set to confocal with an imaging plane of 1.5 µm using a 40x water immersion. There were 197 fields of view captured per well. The channels Alexa 488, Alexa 568 and HOECHST 33342 were used to measure RAD51/H2AX, geminin and nuclear stains respectively. Files were then exported automatically to the WEHI Columbus server (PerkinElmer), where a custom foci analysis pipeline was run using the following settings: Find nuclei was set to method C with a >30 µm2 area threshold and standard morphology properties. Nuclei were then filtered based on an area of 50-150µm2 and a roundness of >0.9 to make the “assay nuclei” group. This group was then further filtered based on a geminin staining intensity mean (Alexa 568) of >800-1500. The “find spots” protocol was then applied, using method C to filter for spots with a mean intensity of >900-2500 within the geminin positive group. The results were plotted using the PRISM7 (GraphPad) software.

*Single nucleotide polymorphism array analysis*

Data were processed using the Genotyping module (v.1.9.4) in GenomeStudio v.2011.1 (Illumina) to calculate B-allele frequencies (BAF) and logR ratios. Copy number changes were estimated using GAP (4), after low-quality probes assessed in the matched normal sample with GenCall (GC) score of <0.7 were removed (where matched normal sample was available). Tumor cellularity was estimated using qpure (5).

*RNA sequencing analysis of public data*

Briefly, adapter sequences were trimmed using Cutadapt (version 1.11) (6) and aligned using STAR (version 2.5.2a) (7) to the GRCh37 assembly with the gene, transcript, and exon features of Ensembl (release 75) gene model. Quality control metrics were computed using RNA-SeQC (version 1.1.8) (8) and expression was estimated using RSEM (version 1.2.30) (9). Data was corrected for library size using counts per million (CPM) and was corrected for differences in RNA composition using trimmed mean of M values (TMM) (10).

**Supplementary References**

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