Investigating BRCAness in Epithelial Ovarian Cancer (EOC) in India to Develop Stratified Surgical and Chemotherapy Options

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Introduction

- Epithelial ovarian cancer (EOC) is associated with poor survival compared to many other solid cancers [1,2].
- EOCs are deficient in homologous recombination DNA repair (50% HR deficient or HRD) known as BRCAness and show improved chemoresponse to PARP inhibitor (PARPi) and platinum as well as better surgical outcomes [3,4].
- The other 50% HR competent (HRC) tumours represent a chemo-resistant population representing an unmet clinical and research need.
- Hyperthermia is a type of cancer treatment in which body tissue is exposed to high temperatures (up to 113°F) by induction of DNA repair dysfunctionality.

Hypothesis

- Developing a functional assay to distinguish between HR-proficient and HR-deficient tumors so that appropriate, effective, tumor-selective chemotherapy can be administered.
- Whether hyperthermia alters the response to chemotherapy-induced DNA damage and whether this mechanism is involved in its sensitizing effect in BRCA competent models of ovarian and other cancers.

Aims & Objectives

- Investigating prevalence of BRCAness in EOC in India with surgical and clinical correlation.
- To develop a preclinical model to test novel chemotherapy options in BRCAness stratified patient tissue samples.

Materials & Methods

Primary cultures were derived from ascetic fluid and tumor tissues from patients with EOCs. EOCs cell lines were also used.

Study Design

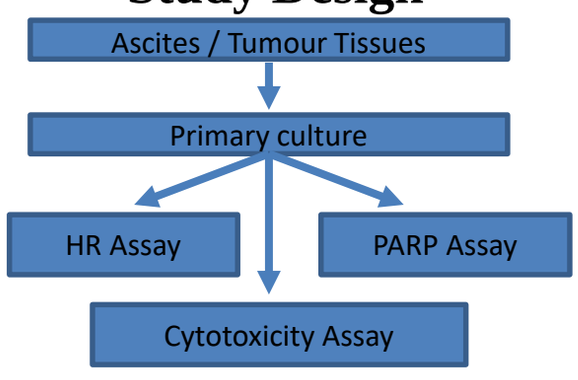

Figure 1: Overall Study design.

Results

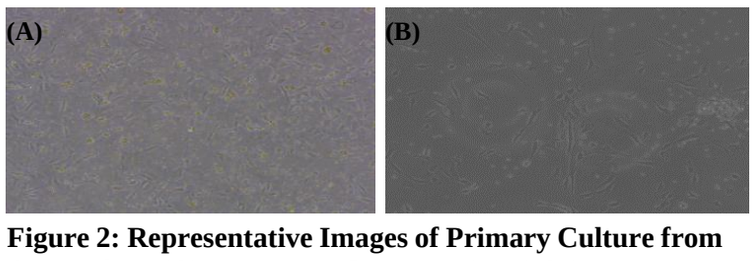

Figure 2: Representative Images of Primary Culture from Ascites fluid (**A**) and Tumor Omentum **(B)**. (20x).

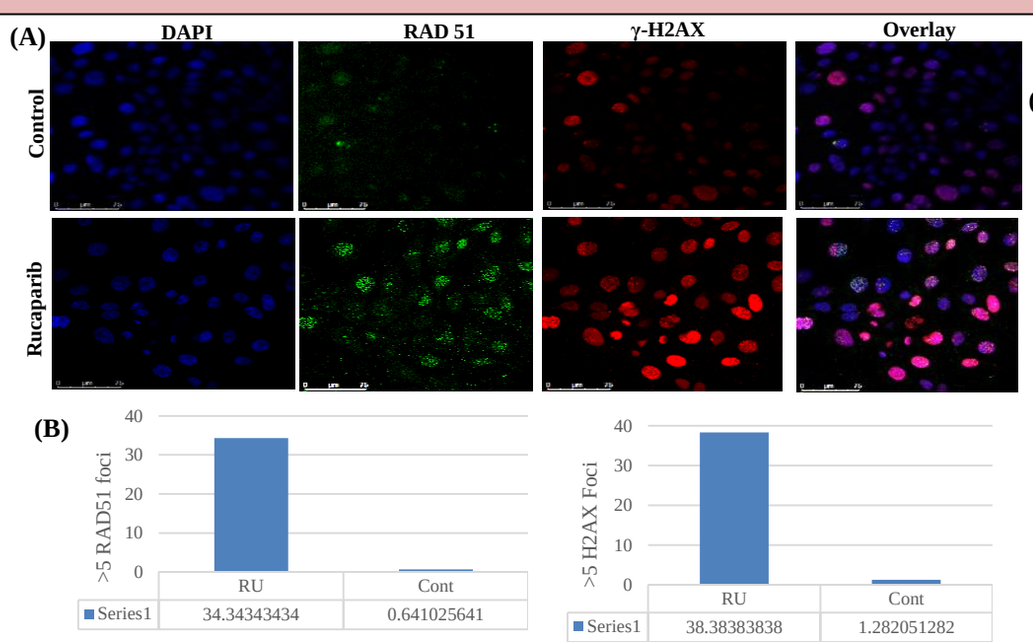

Figure 3: The effects of PARP1-*I* (Rucaparib; 10μM) treatment on UWB+BRCA1 cells are shown. γ-H2AX and Rad51 co-localization (A). Percentages of cells with more than 5 foci per nucleus (B). (20x).

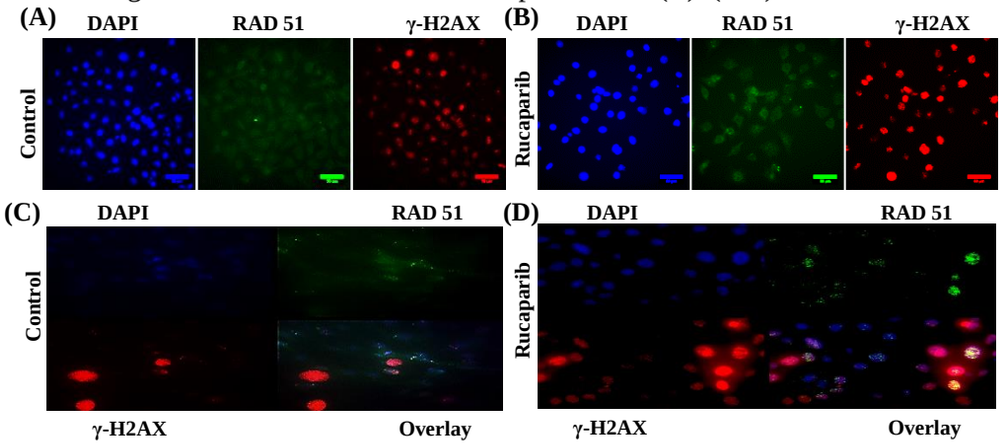

Figure 4: The effects of PARP1-*I* (Rucaparib; 10μM) treatment on VC8 B2 (A & B) and Tumor Omentum (CaOv-233) Control (C) and Rucaparib treatment (D) are shown (representative images). γ-H2AX and Rad51 formation were measured by fluorescence microscopy. (40x).

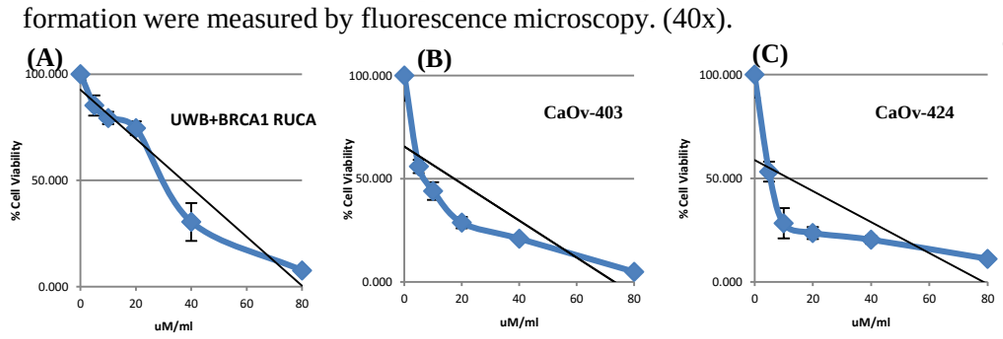

Figure 5: The Cytotoxicity (MTT) Assay: Cytotoxicity effects of PARP1-*I* (Rucaparib) on UWB+BRCA1 (A) and Ascites cells (CaOv-403, CaOv-424; B & C respectively).

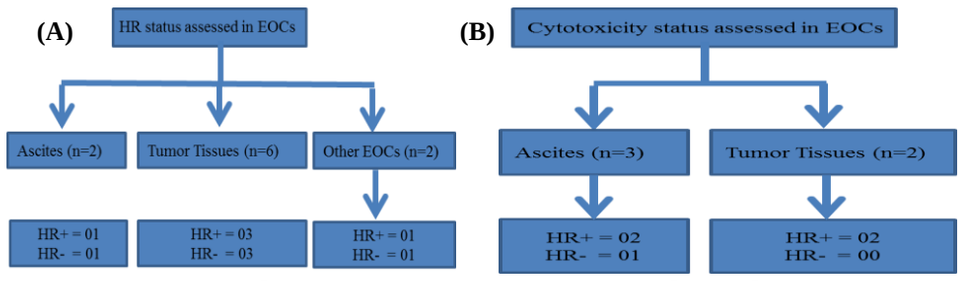

Figure 6: HR (A) and Cytotoxicity assay (B) status and correlation.

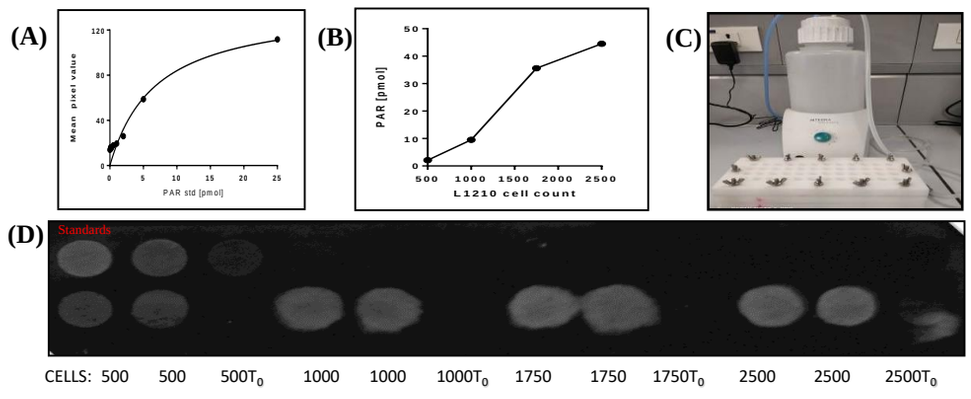

Figure 7: PARP Assay: (A) Standard Curve of purified PAR (B) PARP measured in L1210 cells (C) Manifold and Vacusafe are used in PARP assay (D) Immunoblot developed with purified PAR standard and L1210 cells respectively.

Cells Number	Avg. PAR (Control)	Avg. PAR (+1μM rucaparib)	% inhibition
500 500	40.39	22.98	43.11
2000 2000	166.58	99.475	40.28

Table 1: PARP activity in control V-C8 B2 cells and those pre-treated for 30 min with 1 uM rucaparib determined by measuring PAR formation using 10H antibody after a 6 min reaction in the presence of NAD and oligonucleotide as substrates.

Future Direction

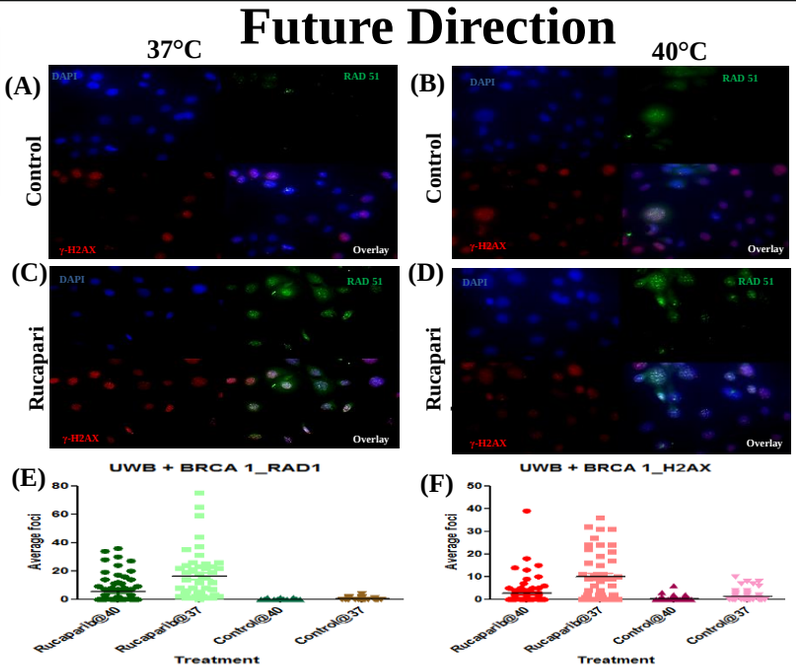

Figure 8: The effects of PARP1-*I* (Rucaparib; 10μM) treatment on UWB+BRCA1 cells at 37°C and 40°C are shown. Control (A & B), Rucaparib treatment (C & D). Number of foci per nucleus from representative images (E & F). (40x).

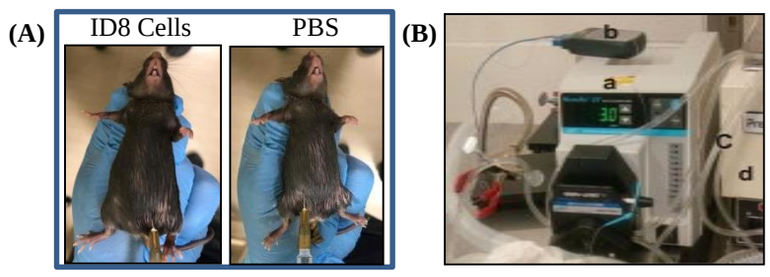

Figure 9: Developing EOC in animal and Hyperthermia: Ovarian cancer can be developed in C57BL/6 by ID8 cells (1385/GO/Re/Bi/S/10/CPCSEA) (A). Hyperthermia technique can be applied by using Masterflex pump (B).

Discussion

- We have shown EOCs can be classified into two groups based on Rad51 foci formation, suggesting that these cells are either HR competent (HRC) or HR deficient (HRD) and it was consistent both in cell lines and in primary cultures.
- This test can be use as a biomarker to direct subsequent therapy.
- Further studies are required to explore that how hyperthermia's attenuation of homologous recombination at 42°C in HRC group.

Conclusion

- HR status can be determined in primary cancer samples by Rad51 focus formation, and this correlates with *in vitro* response to PARP inhibition.
- Use of this assay as a biomarker now needs testing in the setting of a clinical trial.

References

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