Investigating homologous recombination repair and PARP inhibitor sensitivity in lung cancer cell lines

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Background:

- Single strand DNA breaks (SSB) occur in our cells every day. SSB are usually repaired by the Base Excision Repair pathway (BER), which Poly (ADP-ribose) polymerase (PARP) is a key enzyme in.
- If left unrepaired SSB can be turned into double strand DNA breaks (DSB). DSB can be repaired by a process of Homologous Recombination Repair (HRR) (Fig. 1).
- PARP inhibitors (PARPi) can be used to selectively kill cancerous tissue with defective HRR, such as BRCA1/2 defective cells, in a process of synthetic lethality (Fig. 1).
- An exciting area of research is to look at other cancers, other than BRCA1/2 mutated cancers, to see if HRR is functional and whether they are sensitive to PARPi treatment.

Aims:

- To investigate the function of homologous recombination in lung cancer cell lines.
- To determine sensitivity of lung cancer cell lines to PARP inhibitors (PARPi).

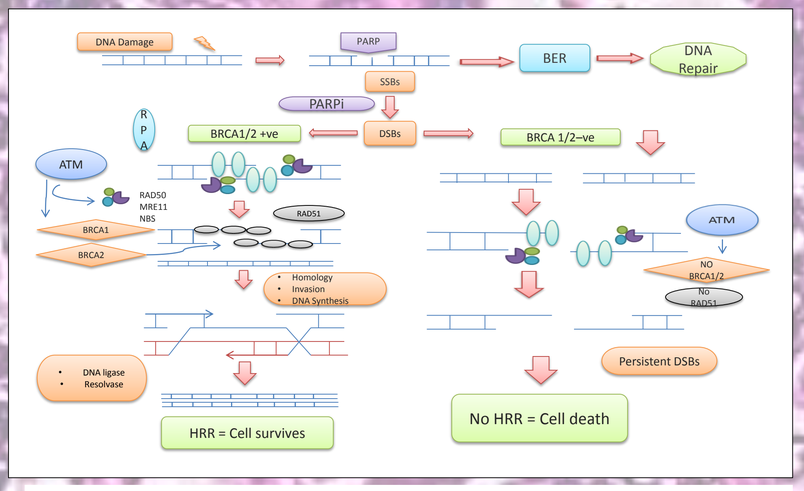


Figure 1. Synthetic lethality; PARP inhibition and Homologous Recombination pathway.

Methods 1: yH2AX and RAD51 focus assay

- Non-small cell lung cancer cell lines, A549 and H522, were grown on coverslips, DNA damage was induced with PARPi treatment.
- Immunofluorescence was used to detect yH2AX and Rad 51 foci. DAPI was used to stain the nucleus (Fig. 2).

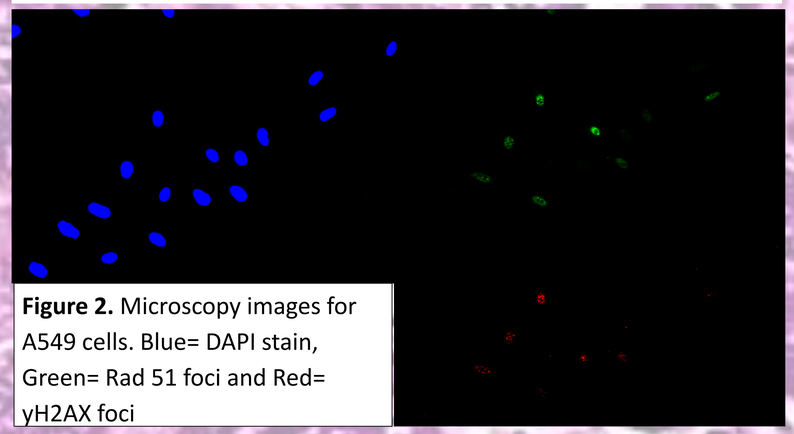
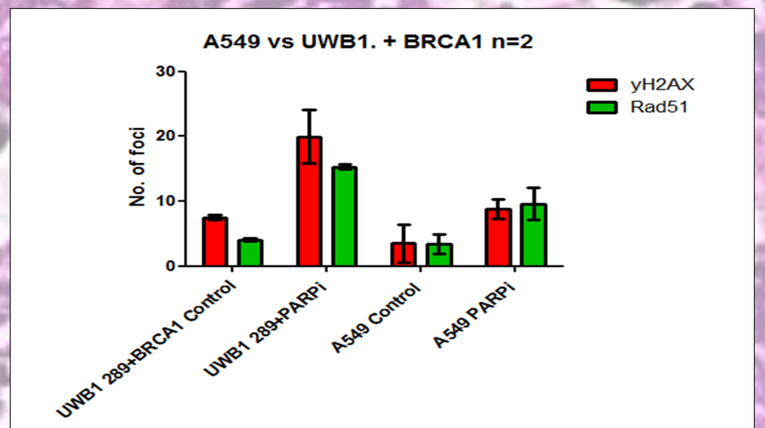


Figure 2. Microscopy images for A549 cells. Blue= DAPI stain, Green= Rad 51 foci and Red= yH2AX foci



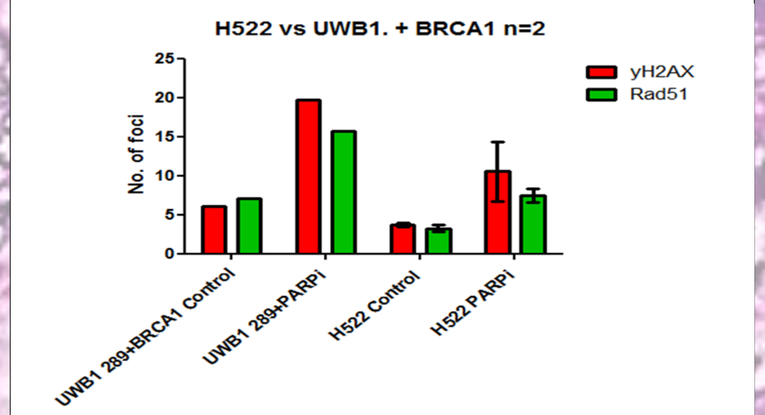


Figure 3. Quantification of yH2AX and RAD51 assay for A549 and H522 cells alongside a positive control cell line; UWB1.289+BRCA1.

Methods 2: DAPI assay

- 1000 cells were seeded into each well of a 96 well plate in duplicate. Following 48 hrs growth one plate was fixed (Day 0, used to monitor growth). Each row of the other plate was treated with increasing concentrations of PARPi
- Cell survival was assessed with DAPI staining.

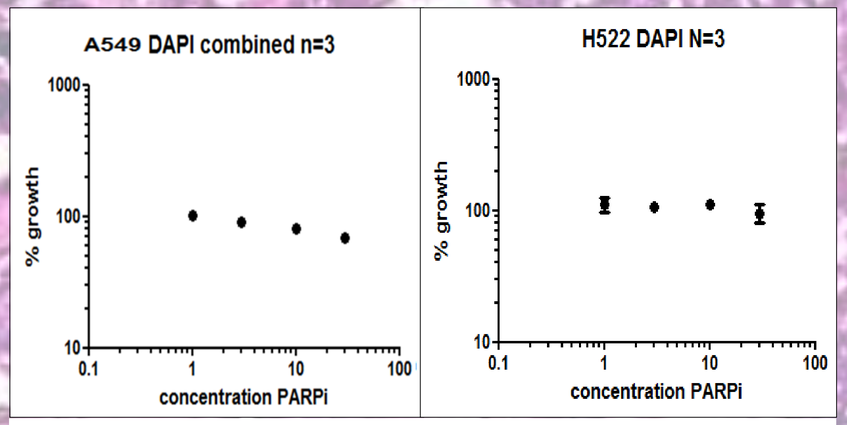


Figure 4. DAPI viability assay of A549 cells (left) and H522 cells (right) following PARPi treatment.

Results and discussion:

- Results of the yH2AX and Rad51 assay showed that the A549 and H522 cells were HRR competent (Fig. 3).
- DAPI assays showed that the cells were insensitive to PARPi as growth did not decrease with rising concentrations of PARPi (Fig. 4). However, comparisons to Day 0 showed that cells did not grow in this time so further investigation is needed.
- Clonogenic assays for cell viability following PARPi treatment were carried out, but the cells did not clone and sensitivity of PARPi could not be measured (data not shown).
- A549 and H522 cell lines would not appear to be suitable for PARP treatment as a monotherapy due to having function HRR.
- More lung cancer cell lines and primary cultures will need to be tested in similar experiments to make a conclusion about the potential for PARPi treatment in lung cancer.