BRCA1/2 MUTATION ANALYSIS IN 43 OVARIAN CELL LINES REVEALS ONLY ONE FUNCTIONALLY DELETERIOUS BRCA1 MUTATION.

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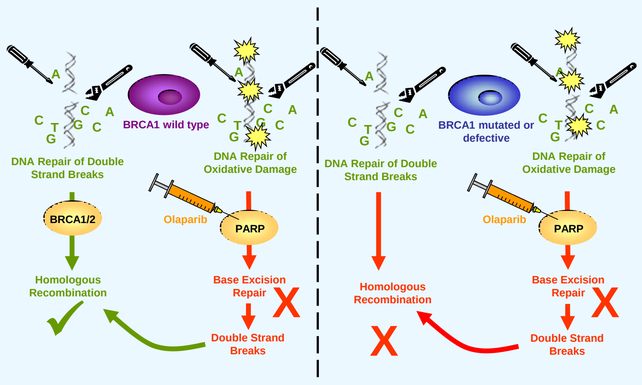
Introduction

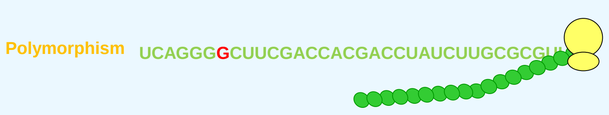
- Germline mutations in DNA repair genes BRCA1/2 increase the risk of developing breast and ovarian cancer.
- Germline BRCA1/2 mutations occur in 8.6-13.7% of unselected epithelial ovarian cancers, somatic BRCA1/2 mutations are also frequent (18.3%).
- BRCA1/2 mutated or dysfunctional cells may be sensitive to parp inhibition by synthetic lethality, the inhibition of two major DNA repair pathways.



Aims of Study

- To screen a panel of 43 ovarian cancer cell lines for BRCA1/2 deleterious mutations and BRCA1 gene methylation.
- To determine if BRCA1 deleterious mutated and methylated cell lines are more sensitive to the parp inhibitors Olaparib and Veliparib.

Types of BRCA1/2 Mutations

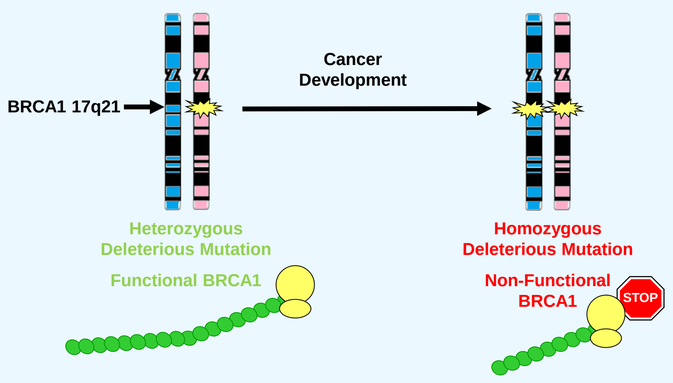


- The majority of mutations in the BRCA1/2 genes are polymorphisms which have no functional impact on the protein.

- Mutations that result in a stop codon cause a truncated non-functional protein.

- Deleterious BRCA1/2 mutations have also been shown to revert in cell lines and cancer patients restoring a functional protein.

Heterozygous or Homozygous?



- A heterozygous BRCA1/2 mutation has no deleterious effect as there is still one functional copy of the gene. BRCA1/2 mutation carriers are heterozygous and the cancer becomes homozygous.

Results – BRCA1/2 Gene Sequencing

- The BRCA1 and BRCA2 genes were sequenced in a panel of 43 ovarian cancer cell lines.
- Only one cell line had a functionally deleterious mutation in BRCA1 (SNU-251).
- Two cell lines had heterozygous mutations in BRCA1 (IGROV-1) and BRCA2 (OC316), but these have no functional impact on the protein.

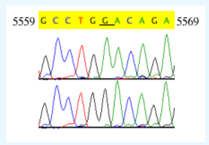


Figure 1A
Top: SNU-251 showing homozygous deletion W1815X (5564G>A)
Bottom: BRCA1 Wild type sequence

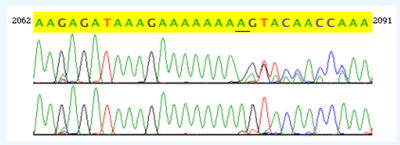


Figure 1B
Top: IGROV-1 showing heterozygous deletion - 2080delA
Bottom: BRCA1 Wild type sequence

- Two cell lines had deleterious mutations as well as an additional reversion mutation which has restored the protein back to wild type BRCA1 (UPN-251) and BRCA2 (PEO1).

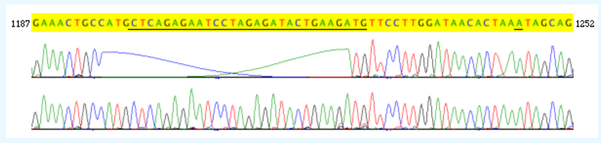
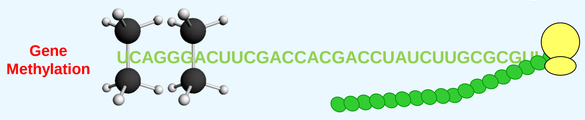


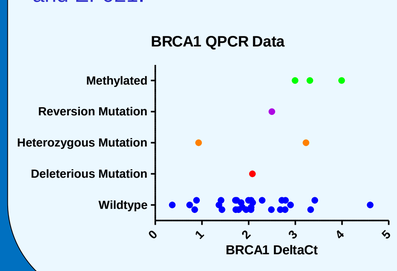
Figure 1C
Top: UPN-251 showing homozygous deletions - 1199del29 + 1246delA Reversion Mutation
Bottom: BRCA1 Wild type sequence

Figure 1D
a)PEO1 - 5192A>T + 5193C>G Y1655L Reversion Mutation
b)PEO4 - 5193C>T Y1655Y
c)PEO6 - 5193C>T Y1655Y
d) BRCA2 Wildtype sequence

Results - BRCA1 Gene Methylation

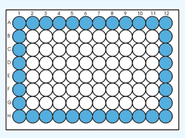


- BRCA1 gene methylation was examined in the panel of 43 ovarian cancer cell lines. Three cell lines were found to be methylated: A1847, OVCAR8 and EF021.

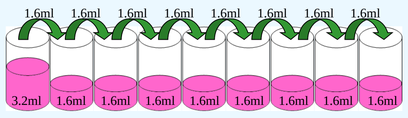


- The methylated cell lines have a corresponding decrease in BRCA1 mRNA expression.
- The SNU-251 cells have similar BRCA1 expression levels to wild type cells. This is due to the location of the QPCR primers. The SNU-251 cell line's deleterious mutation is at the very tail end of the gene sequence.

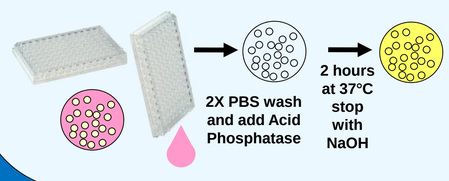
Method – Cytotoxicity Assays



Day 1 – 1 x 10^3 cells per well were plated into the 60 central wells of a 96-well plate. Cells were then allowed to attach overnight.



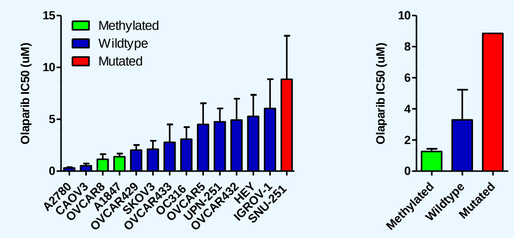
Day 2 – Serial dilutions of Olaparib and Veliparib were added to the cells in triplicate wells.



Day 7 – Cells were stained with phosphatase substrate and absorbance read at 412nm.

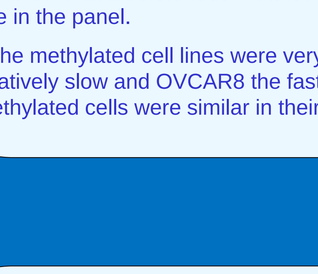
Results - Cytotoxicity of Parp Inhibitors

- A smaller panel of 14 cell lines was chosen to investigate the impact of BRCA1/2 dysfunction on sensitivity to parp inhibitors. Eleven wild-type cell lines were compared to two methylated cell lines (A1847 and OVCAR8) and the deleterious mutant (SNU-251).



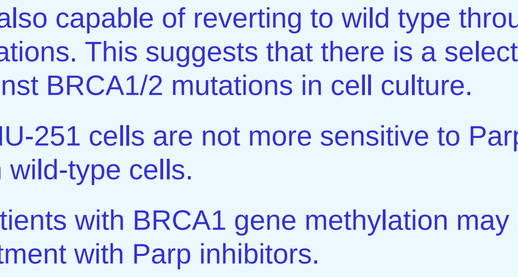
- Unexpectedly, the SNU-251 cell line with the deleterious mutation in BRCA1 was the most resistant to Olaparib of the panel.
- The BRCA1 methylated cell lines were relatively sensitive to Olaparib. Olaparib may be more useful in treating patients with BRCA1 gene methylation rather than deleterious mutation.

- A similar trend was observed for Veliparib, SNU-251 was relatively resistant, and the methylated cell lines tended to be sensitive.



- Some correlation was seen between resistance to Olaparib and resistance to Veliparib in the cell line panel.

Results – Growth Rate of Cell Lines



- The SNU-251 deleterious mutated cells were the slowest growing cell line in the panel.
- The methylated cell lines were very different in their growth rates, A1847 relatively slow and OVCAR8 the fastest. However, on average the methylated cells were similar in their growth to the wild type cells.

Conclusions

- BRCA1/2 deleterious mutations are much rarer in the cell line panel 1/43 (2.3%) compared to ovarian cancer patients.
- BRCA1 deleterious mutated cells (SNU-251) grow slower in cell culture than wild type cells. BRCA1/2 mutated cells are also capable of reverting to wild type through additional mutations. This suggests that there is a selective pressure against BRCA1/2 mutations in cell culture.
- SNU-251 cells are not more sensitive to Parp inhibition than wild-type cells.
- Patients with BRCA1 gene methylation may benefit from treatment with Parp inhibitors.