RESISTANCE RELATIONSHIPS BETWEEN PLATINUM AND PARP-INHIBITORS IN OVARIAN CANCER.

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Kinds of BRCA1/2 Mutations

**Wild Type**
UCAGGGACUUCGACCACGACCUAUCUUGCGCGU
- Full Length Functional Protein

**Polymorphism**
UCAGGGGCUUCGACCACGACCUAUCUUGCGCGU
- Full Length Functional Protein

**Deleterious Mutation**
UCAGGGACUUCGACCACGACCUAUCUUGCGCGU
- Truncated Non-Functional Protein (STOP)

**Reversion Mutation**
UCAGGGACUUCGACCACGACCUAUCUUGCGCGU
- Full Length Functional Protein
Heterozygous or Homozygous?

BRCA1 17q21

Heterozygous
Deleterious Mutation
Functional BRCA1

Cancer Development

Germline

Tumour

Homozygous
Deleterious Mutation
Non-Functional BRCA1

STOP
Germline or Somatic?

BRCA1 17q21

Cancer Development

Germline Tumour

Heterozygous Deleterious Mutation

Functional BRCA1

• Test DNA from blood or other non-tumour tissue to assess germline

Homozygous Deleterious Mutation

Non-Functional BRCA1

• Testing the tumour or cell lines made from tumors is the combined result of germline and somatic mutations
BRCA1, Cisplatin and PARP Inhibition

BRCA1 wild type

Cisplatin

DNA Repair of Cisplatin Adducts

BRCA1/2

Homologous Recombination

Nucleotide Excision Repair

Double Strand Breaks

Olaparib

PARP

Base Excision Repair

BRCA1 mutated or defective

Cisplatin

DNA Repair of Cisplatin Induced Oxidative Damage

Olaparib

PARP

Double Strand Breaks

Base Excision Repair

Homologous Recombination

Nucleotide Excision Repair
Aims of Study

• To screen a panel of 41 ovarian cancer cell lines for BRCA1/2 deleterious mutations and BRCA1 gene methylation.

• To determine if BRCA1/2 deleterious mutated and methylated cell lines are more sensitive to platinum and parp inhibitor chemotherapy.

• To determine if any BRCA1/2 wild-type cells are highly sensitive or resistant to platinum and parp inhibitors.

• To discover gene expression profiles of platinum and parp inhibitor resistance and sensitivity and determine any overlap between these profiles.
BRCA1/2 Gene Sequencing

• The full length of BRCA1 and BRCA2 genes were sequenced in a panel of 41 ovarian cancer cell lines.

• Only one cell line had a functionally deleterious mutation in BRCA1 (SNU-251).

• Seven cell lines had heterozygous mutations in BRCA1 (IGROV-1) or BRCA2 but these have no functional impact on the protein.

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

Top: IGROV-1 showing heterozygous deletion - 2080delA
Bottom: BRCA1 Wild type sequence
BRCA1/2 Gene Sequencing

- 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).

Top: UPN-251 showing homozygous deletions - 1199del29 + 1246delA Reversion Mutation
Bottom: BRCA1 Wild type sequence
• BRCA1 gene methylation was examined in the panel of 41 ovarian cancer cell lines. Two cell lines were found to be methylated: A1847 and OVCAR8.

• 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 at the very tail end of the gene sequence.
Frequency of BRCA1/2 mutations

- Represents both germline and somatic mutations

Unselected Ovarian Cancer Cell Lines from n=33 Patients
- BRCA1/2 Deleterious Mutations 3%
- Wild Type 97%

Unselected Invasive Ovarian Tumours (Literature 1-3)
- BRCA1/2 Deleterious Mutations 8.6-13.7%
- Wild Type 91.4-86.3%

- Represents germline mutations only. Studies including somatic have reported 18.3% mutated in ovarian tumours (4)

Selective Pressure Against BRCA1/2 Mutations?

• Primary culture protocols usually involve physical disruption of the tissue, enzymatic digestion and selection of attached colonies in tissue culture.

• The more robust the cell the more likely it is to survive the process, cells without the full complement of DNA repair pathways, such as BRCA1/2 mutants are likely to be at a disadvantage.

• We examined the growth rate of 19 cell lines from the ovarian cell line panel and the deleterious mutant SNU-251 had the slowest growth rate.
Two cell lines in the cell panel had deleterious mutations and an additional reversion mutation (UPN-251) and (PEO1), also suggesting that there is a selective pressure against BRCA1/2 mutations in culture.

This effect has been observed in drug resistant cell lines, as well as in cancer patients post chemotherapy.

Sakai et al 2008 Nature 451(7182):1116-1120
The high rate of heterozygous mutations was unexpected, these have no functional impact on the BRCA1/2 protein. No LOH was observed at the BRCA1/2 locus in these cells.

This is a much higher rate than observed in clinical ovarian cancer. These may represent heterogeneity within a BRCA1/2 tumour with the heterozygous cells selected for in culture.
Cytotoxicity of Cisplatin

• A smaller panel of 20 cell lines were chosen to investigate the impact of BRCA1/2 dysfunction on sensitivity to platinum. Seventeen wild-type cell lines were compared to the methylated cell lines (A1847 and OVCAR8) and the deleterious mutant (SNU-251).

![Graph showing Cisplatin IC50 (µM) for different cell lines.](image-url)
Biomarkers of Cisplatin Resistance

- Affymetrix whole genome arrays were performed on the panel of cell lines. Gene expression was compared between:
  - Relatively Cisplatin Sensitive OAW42, CAOV3 and IGROV-1
  - Relatively Cisplatin Resistant HEY, OVCAR432 and OVCAR433

- When comparing the relatively cisplatin resistant or sensitive cell lines on a whole genome basis they do not cluster together distinct from the other cell lines.
Biomarkers of Cisplatin Resistance

• 305 genes were significantly different between the two groups of cell lines. These were analysed by Ingenuity Pathway Analysis. Pathways with a significant number of altered genes were:-
  • Integrin Signalling
  • Ephrin Receptor Signalling
  • IL8 Signalling
  • Glutathione Pathway
Biomarkers of Cisplatin Resistance

• 305 genes were significantly different between the two groups of cell lines. These were analysed by Ingenuity Pathway Analysis. Pathways with a significant number of altered genes were:
  • Integrin Signalling - ↑ITGA6 (alpha integrin) → ↑AKT3
  • Ephrin Receptor Signalling - ↑EPHA4 → ↑AKT3
  • IL8 Signalling - ↑IL8 → ↑AKT3

• Activation of the Akt/mTOR pathway prevents cisplatin-induced apoptosis in ovarian cancer cells

Absence of “Usual Suspects”

- However, this may be due to us comparing untreated cell lines rather than genes responsive to cisplatin treatment
Glutathione and Cisplatin Resistance

- The platinum-glutathione conjugate can no longer bind to DNA hence removing the toxic effect of the drug.

- Any increase in activity the glutathione pathway, synthesis, recycling or conjugation to cisplatin can potentially mediate cisplatin resistance.

- However, we see no change in glutathione reductase or synthetase.
Glutathione and Cisplatin Resistance

- Glutathione functions to detoxify xenobiotics such as cisplatin by a conjugation reaction. The platinum-glutathione conjugate can no longer bind to DNA hence removing the toxic effect of the drug.

- In contrast, we see a decrease in GSTM1, GSTM2, and GSTM3 associated with cisplatin resistance.
Glutathione and Cisplatin Resistance

- Glutathione is a tri-peptide which is synthesised within the cell from the amino acids glutamate, cysteine and glycine.

- We see an increase in aminopeptidase associated with cisplatin resistance which may increase the available building blocks for glutathione synthesis.
• Unexpectedly, the SNU-251 cell line with the deleterious mutation in BRCA1 was one of the most resistant cell lines to olaparib of the panel.

• On average the BRCA1 methylated cell lines A1847 and OVCAR8 were relatively sensitive to Olaparib.
Cytotoxicity of Veliparib

- A similar trend was observed for Veliparib, SNU-251 was relatively resistant, and in a one week assay but not in a 2 week assay.
- The methylated cell lines tended to be sensitive to Veliparib.
Cytotoxicity of Olaparib vs Veliparib

- Relatively Veliparib Resistant: SKOV3, UPN-251, OVCAR420
- Relatively Veliparib Sensitive: A2780, ES-2, OVCA433
- Relatively Olaparib Resistant: OVCAR432, HEY, IGROV-1, OVCAR420
- Relatively Olaparib Sensitive: A2780, CAOV3, ES-2, HOC1

This suggests a similar but slightly divergent mechanism of resistance where a cell line specialises more in resistance to one agent over the other.
Biomarkers of Olaparib Resistance

- Affymetrix whole genome arrays were performed on the panel of cell lines. Gene expression was compared between:
  - Relatively Olaparib Sensitive A2780, CAOV3, ES-2, HOC-1
  - Relatively Olaparib Resistant HEY, IGROV-1, OVCAR420 OVCAR432

- When comparing the relatively olaparib resistant or sensitive cell lines on a whole genome basis they do not cluster together distinct from the other cell lines.
Biomarkers of Veliparib Resistance

- Affymetrix whole genome arrays were performed on the panel of cell lines. Gene expression was compared between:
  - Relatively Veliparib Sensitive A2780, ES-2, OVCAR433
  - Relatively Veliparib Resistant OVCAR420, SKOV3, UPN-251

- When comparing the relatively veliparib resistant or sensitive cell lines on a whole genome basis they do not cluster together distinct from the other cell lines.
Biomarkers of Parp Inhibitor Cross Resistance

• 25 genes were significantly different between the olaparib resistant and sensitive cell lines.
• 151 genes were significantly different between the veliparib resistant and sensitive cell lines.
• These were analysed by Ingenuity Pathway Analysis. The top pathways were different for Olaparib and Veliparib, most were general cancer pathways. Of interest:-
  Olaparib – G2/M DNA Damage Checkpoint Regulation
  Veliparib - Pyrimidine De Novo Biosynthesis
Biomarkers of Parp Inhibitor Cross Resistance

- Only 1 gene was significantly differentially expressed in both Olaparib and Veliparib resistant cell lines PLCL2 - phospholipase C-like 2
- Phosphodiesterases that cleave the polar head groups from inositol lipids
- Thought to activate the Src pathway.

- PLCL2 was increased in the sensitive cell lines. Also found in the NCI-60 panel as a general marker of drug potency.
- However, the overall gene expression profiles are similar suggesting a common mechanism of resistance between the two agents but some specificity in a different subset of genes.
Cytotoxicity of Cisplatin vs Olaparib

- Relatively Olaparib Resistant: OVCAR432, HEY, IGROV-1, OVCAR420
- Relatively Cisplatin Resistant: OVCAR432, HEY, OVCAR433
- Relatively Olaparib Sensitive: A2780, CAOV3, ES-2, HOC1
- Relatively Cisplatin Sensitive: PEO1, OAW42, CAOV3, IGROV-1

Correlation R = -0.0977 p = 0.690

↓AKT3
↑AKT3
Cytotoxicity of Cisplatin vs Veliparib

- Cisplatin appears active in veliparib-resistant cell lines.
- Veliparib somewhat active in cisplatin-resistant cell lines.
- Potential for combination treatments of these agents for ovarian cancer (toxicity permitting).
Conclusions

• Deleterious BRCA1/2 mutations are rare in the panel of ovarian cancer cell lines we have studied 2.6%. There appears to be selective pressure against BRCA1/2 mutations in cell culture.

• BRCA1 mutated and methylated cell lines are sensitive to platinum and parp inhibitor chemotherapy. There are cohorts of BRCA1/2 wild-type cell lines that are sensitive to platinum and parp inhibitors.

• The AKT pathway may be a ‘global’ pathway suitable for use as a broad platinum resistance biomarker at the gene level. Rather than using the ‘usual suspects’
Please come and see poster presentations

- Developing platinum and taxane resistant ovarian cancer cell lines: Investigating the role of BRCA1.
- Collateral sensitivity to cisplatin in KB-8-5-11 is confluence dependant.