Drug Sensitivity of a Spontaneously Immortalised Human Ovarian Cancer Cell Line

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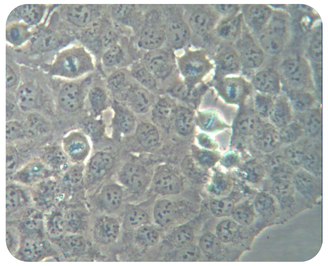
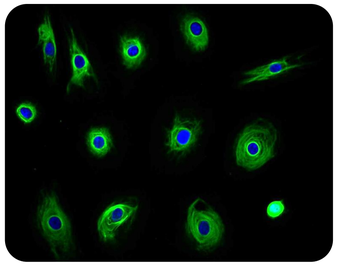
Introduction

- Between 5 and 10% of ovarian cancers are clear cell carcinomas (CCC) which generally respond poorly to chemotherapy.
- Cells cultured from the ascitic fluid of a patient with CCC spontaneously immortalised in the lab and established the cell line NUCOLL43.
- NUCOLL43 cells are epithelial as shown by morphology and pan-cytokeratin assay and show many similarities with the original tumour so they are a good model to study CCC.



Brightfield microscopy image of NUCOLL43 at 20x magnification.



Pan-cytokeratin assay showing NUCOLL43 is of epithelial origin.

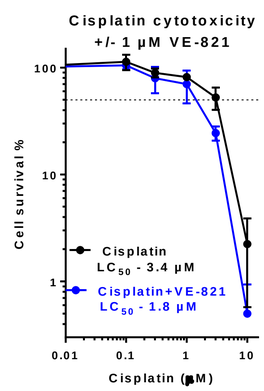
- The aim was to test the sensitivity of NUCOLL43 to different therapies: cisplatin (standard chemotherapy) with and without a novel ATR inhibitor (VE-821), radiotherapy +/- a PARP inhibitor (rucaparib) and a PI3K/mTOR inhibitor (NVP-BEZ235).

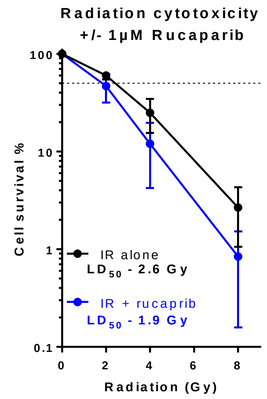
Methods

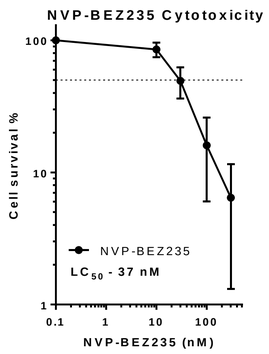
- Cells were seeded at low density for colony formation, treated with the various drugs (and combinations) for 24 hr and the number of colonies formed after 7 days counted to work out the concentration of drug needed to kill 50% of the cells (LC_{50}).
- The ability of NVP-BEZ235 to inhibit PI3K and mTOR was determined by measuring the phosphorylation of AKT and 4E-BP1 by Western Blot and performed in triplicate.

Results

Survival of NUCOLL43 cells after treatment with increasing drug concentrations and radiation doses



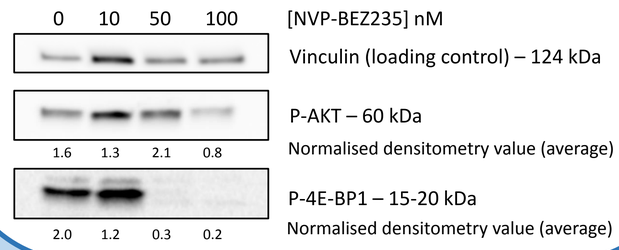




The number of colonies that grew, i.e. the survival of cells (y axis) was reduced with increasing concentrations of drug or doses of radiation.

Adding the ATR inhibitor, VE-821, increased the killing effect of cisplatin. Also the PARP inhibitor, rucaparib, made radiation more effective at killing cells.

Western Blot of NUCOLL43 treated with NVP-BEZ235



Discussion

- The concentration of cisplatin needed to kill 50% of the cells (LC_{50}) was 3.4 µM. This is higher than in most cell types, indicating that NUCOLL43 is resistant to cisplatin. The ATR inhibitor VE-821 made them approximately twice as sensitive (LC_{50} reduced to 1.8 µM).
- The dose of radiation needed to kill 50% of the cells (LD_{50}) was 2.6 Gy, which is similar to other cell lines and the radiosensitivity was increased by the PARP inhibitor, rucaparib (LD_{50} reduced to 1.9 Gy).
- NUCOLL43 cells were quite sensitive to NVP-BEZ235 with the concentration needed to kill 50% of the cells being only 37 nM, which is lower than has previously been reported for clear cell ovarian cancer (albeit using different methodology[1]).
- NVP-BEZ235 reduced the expression of p-4E-BP1 indicating inhibition of the mTOR pathway and in turn should reduce cancer growth. The results for p-AKT (as a result of PI3K inhibition) are not as convincing therefore this may be repeated as part of future work.
- These results are being used in conjunction with others to fully characterise NUCOLL43 so that it can be used as a CCC model by the scientific community.

Acknowledgements/References

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- 1) T. Oishi HI, A. Kudoh. The PI3K/mTOR dual inhibitor NVP-BEZ235 reduces the growth of ovarian clear cell carcinoma. Oncology Reports. 2014;32:553-8.