Modeling Resistance and Sensitivity to PRRT

Kelly Waldeck (1), Jessica Van Zuylekom (1), Benjamin Blyth (1, 2), Carleen Cullinane (1, 2), Andrew Pattison (3), Aidan Flynn (3), Richard Tothill (3), Rodney J. Hicks (1, 2, 4).

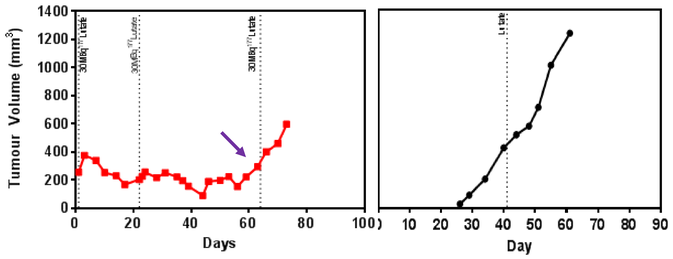
(1) Molecular Imaging and Targeted Therapeutics Laboratory, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia, (2) Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Victoria, Australia, (3) Department of Clinical Pathology and Centre for Cancer Research, University of Melbourne, Melbourne, Victoria, Australia, (4) Division of Cancer Imaging, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

The development of resistance is a common reason for therapy failure, and ways to overcome resistance a significant area of continual research. Peptide receptor radionuclide therapy (PRRT) for neuroendocrine tumours (NET) is now routine, however cures remain rare, as some NET patients are inherently resistant to PRRT, while most develop resistance after initial success. We hypothesise that resistance to PRRT is a manifestation of a general radiation resistance phenotype, mediated through enhanced recognition and repair of radionuclide-induced DNA damage, rather than simply loss of the PRRT target (in this case, the somatostatin receptor type 2 (SSTR2). The Aims of our research are therefore:

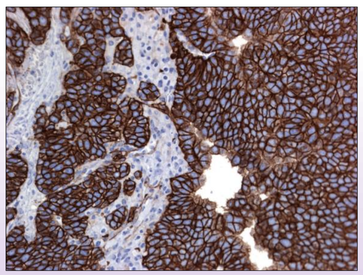
1: To identify genes that may contribute to the development of resistance to PRRT

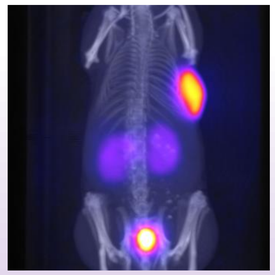
2: To identify genes that play a role in sensitivity to PRRT

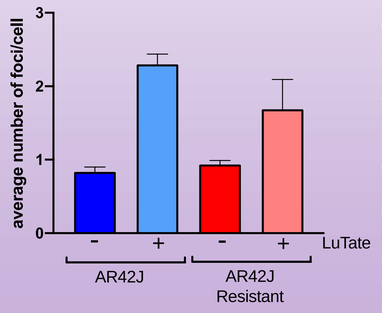
1: Identifying Genes involved in the Development of Resistance



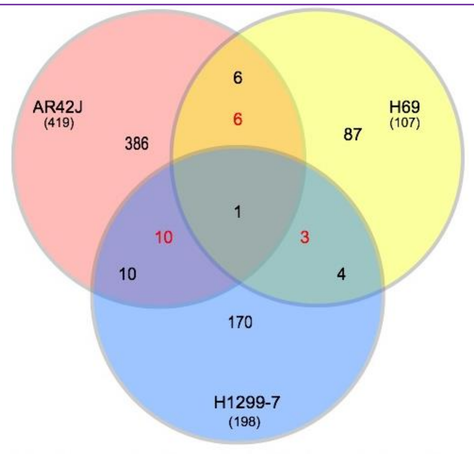
When we treated mice bearing AR42J xenografts with 30 MBq LuTate (example mouse shown above, left), the tumour stops growing and begins to regress. A second dose shows a similar response to LuTate. However, on a third challenge (see arrow), the tumour volume continues to grow at the same rate. When this tumour is re-implanted into a new host and challenged with LuTate (above, right), it continues to show a resistant phenotype, and this tumour was then used to establish a 'LuTate-resistant AR42J' cell line which was then shown to also produce LuTate-resistant xenografts in subsequent hosts (data not shown). Retention of SSTR2 expression in these LuTate-resistant xenografts was confirmed by immunohistochemistry and GaTate PET imaging (below), indicating that loss of the receptor was not the mechanism of resistance in this model.







Showing that LuTate was still effective in damaging DNA in the resistant cell line, gamma-H2Ax staining in tumours 72hr after LuTate challenge showed no significant difference in the levels of accumulated DNA double-strand breaks (left). We have used this process of generating LuTate-resistant tumours and cell lines, in three cell lines, establishing a panel of tools to explore mechanisms of both resistance and sensitivity, and assess drug combinations.

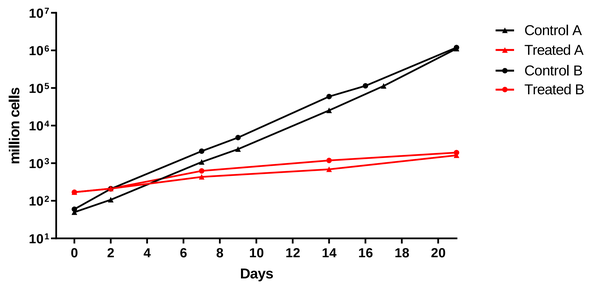


Red numbers - genes altered in same direction (either increased or decreased)

Furthering the analysis of the resistant cell lines, we have examined changes to gene expression at the RNA level, through single cell RNA-sequencing technologies. Preliminary analysis of this data shows that there are 40 genes that show differential expression between their parental and resistant pairs in at least two of the models (above). When this data is expanded to look at non-significant levels of expression eight genes are identified that show a trend to similar alterations across all three resistant models. The table below shows the log-fold change in expression of these 8 genes in the resistant cell lines (as relative to their parental line), with red indicating an increase in expression and blue a decrease.

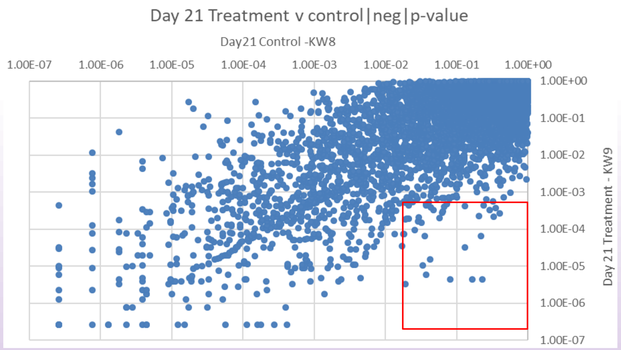
	H69	H1299-7	AR42J
S100A16	1.2	0.6	1.3
GSTP1	1.1	0.5	0.4
GADD45A	0.4	0.4	2.1
ACTB	0.3	0.6	0.5
HNRNPA1	-0.1	-0.5	-1.5
SLC25A3	-0.2	-0.7	-0.6
EEF2	-0.2	-0.5	-1.1
EIF4B	-0.2	-0.7	-0.9

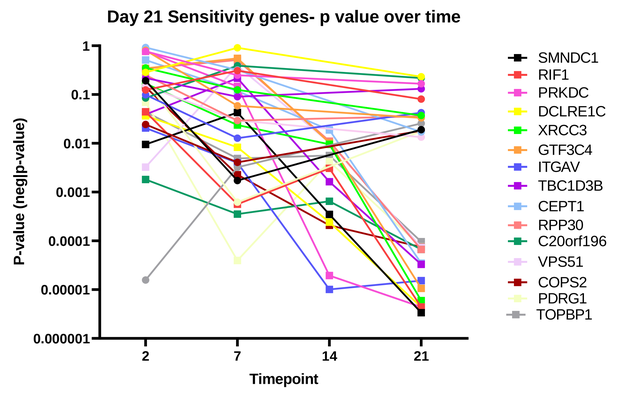
2: Identifying Genes involved in Sensitivity to PRRT



50MBq suspension = 5MBq/mL

As a second approach for the identification of genes involved in both resistance and sensitivity to LuTate, we have used an unbiased whole genome knockout CRISPR screen. As seen above, treatment with 5MBq/mL LuTate resulted in strong growth inhibition in the H1299-7 cells. Upon sequencing, at Day 21 post treatment, we were able to identify a selection of genes that have, when knocked out, resulted in sensitivity to LuTate (indicated by the red box in the plot below).





Taking the top 'sensitivity' gene hits from the CRISPR screen we have tracked the p-value of these genes over time, looking for genes that progressively result in the sensitivity observed at Day 21 (above), and then using these genes looked for any common pathways (below). As predicted in our hypothesis, pathways and genes involved in DNA damage repair were amongst the top hits for sensitivity to LuTate.

GO biological process complete	Number of Genes from CRISPR dataset	over/under represented	Fold Enrichment	P-value
Telomere maintenance in response to DNA damage	2	+	> 100	3.17E-05
Double-strand break repair via nonhomologous end joining	3	+	69.5	1.16E-05
Non-recombinational repair	3	+	63.18	1.53E-05
Telomere maintenance	4	+	57.32	6.77E-07
Telomere organization	4	+	55.6	7.62E-07
Double-strand break repair	4	+	29.73	8.58E-06
DNA repair	6	+	16.13	9.95E-07
DNA metabolic process	6	+	11	8.91E-06
Cellular response to DNA damage stimulus	6	+	10.58	1.11E-05

Conclusions and Future Work

- Resistance to LuTate can be mediated through mechanisms unrelated to SSTR2 expression. With SSTR2 expression unaltered, and gamma H2Ax foci formation in response to LuTate unaltered in our resistant cell lines.
- Eight genes have been identified as showing the same trend in gene alteration in all three resistant cell lines.
- The top pathway hits from the CRISPR screen, for sensitivity to PRRT, are all related to DNA damage repair.
- Analysis is continuing in the Resistant cell lines and tumours, with the expression analysis extending to look at genes involved in the DNA damage repair pathways, and tumours being assessed through Whole Genome Sequencing and further in vivo response experiments.
- The CRISPR screen will be repeated in a second cell line to further the significance of any sensitivity genes identified, with the analysis extended to identifying genes involved in resistance. This data will then be cross-compared with the resistant cell line data and identified targets will be validated.
- And finally, gene targets identified as resulting in sensitivity to LuTate will be used in the Resistant lines as a mechanism to hopefully overcome the LuTate resistance.

Acknowledgements

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