Identifying mechanisms of sensitivity and resistance in response to LuTATE PRRT.

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Background/Significance to NET

Peptide receptor radionuclide therapy (PRRT) using Lu-177 DOTA-octreotate (LuTate) for neuroendocrine tumours (NET) is now an approved treatment in many countries. However, primary and secondary resistance limit its effectiveness or durability. We hypothesise that resistance to LuTate PRRT is mediated through enhanced recognition and repair of radionuclide-induced DNA damage - rather than loss of the PRRT target, somatostatin receptor-type 2 (SSTR2). DNA-damage repair (DDR) pathways are critical for sensing and repairing radiation-induced DNA damage and ultimately directing cell fate. Accordingly, DDR inhibitors could provide for clinically relevant treatment options. Supporting our hypothesis, we have previously shown that the combination of a PARP inhibitor (limiting repair of single-strand DNA breaks) with PRRT improved outcomes in animal models (Cullinane et al, 2020). Additionally, we have shown that generating resistance through repeated LuTATE treatment in animal models does not result from a loss of the SSTR2 receptor. This study is designed to further investigate mechanisms of sensitivity and resistance to PRRT through use of an unbiased whole-genome wide CRISPR screen, in the hope of identifying gene targets to that might inform novel combination therapies for NET patients.

Materials and Methods/Experimental Approach

In order to identify genes that modify sensitivity or confer resistance to LuTATE treatment, we undertook an unbiased genome-wide CRISPR-knockout screen using the H1299-7 cell line, a NSCL cancer line genetically engineered to overexpress SSTR2. Validation of identified targets was undertaken using single gene CRISPR knockout in this cell line, followed by treatment with LuTATE at increasing doses to generate a dose-response curve. SSTR2 expression and LuTATE retention within the single gene knockout cell lines was also examined. Further validation of the sensitivity gene target, DNA-PK, was carried out in both *in vitro* and *in vivo* combination experiments using the DNA-PK inhibitor nedisertib in combination with LuTATE PRRT.

Results

The most significant pathway conferring PRRT sensitivity within the genome-wide CRISPR screen was the DDR pathway. From the identified targets, we first focussed on DNA-PK, the most significant hit. *In vitro* LuTATE response curves showed knock-out of DNA-PK significantly enhanced sensitivity to LuTATE therapy. Additionally, the sensitivity seen with genetic loss of DNA-PK could be replicated phenotypically, both *in vitro* and *in vivo*, through the use of a DNA-PK inhibitor, nedisertib, in a dose-dependent manner. Treatment of H1299-7 xenografts with combination of nedisertib and LuTATE significantly increased survival over either treatment alone. While no significant pathways were identified in the resistance arm of the screen, several interesting candidates were identified. Focussing on one of these, Beta-Arrestin 2, we have validated the LuT resistance observed in *in vitro* dose-response curves. As Beta-Arrestin 2 has been shown to localise with SSTR2 and is believed to play a role in SSTR2 receptor trafficking and recycling, we assessed the impact of loss of Beta-

Arrestin 2 on both SSTR2 expression and LuTATE retention. H1299-7 cells with knock-out of Beta-Arrestin 2 show no alteration to SSTR2 expression or localisation by IHC, however retention of LuTATE within these cells does seem to be reduced.

Conclusions

DNA-PK knock-out or pharmacologic inhibition confer significant sensitivity to LuTATE both *in vitro* and *in vivo*. This data suggests that DNA-PK inhibitors may be an important tool in improving response rates to LuTATE. Studies looking at the resistance gene target Beta-Arrestin 2 suggest that loss of this gene may alter the ability of LuTATE to bind or be retained within the cells, providing a possible mechanism of resistance. Studies are continuing to further elucidate the mechanisms of resistance observed, and to further validate and assess the potential of DNA-PK and other DDR inhibitors as valid targets for overcoming resistance to PRRT.

Lay Abstract

Resistance, either immediate or developed during cancer treatment, is a common reason for therapy failure, and is a focus of continual research. The use of peptide receptor radionuclide therapy (PRRT) for neuroendocrine tumours (NET) is now approved in many countries but responses vary with some patients failing to benefit from initial treatment, whereas others stop responding despite earlier sensitivity. In the laboratory, we have used a technology called CRISPR to identify genes that might be important for response to PRRT in NET. Two genes, in particular, stood out as being important to cell survival following exposure to PRRT. The first is DNA-PK, which is a gene in a pathway involved in recognizing and repairing the damage done to DNA by radiation. We have shown that inactivating DNA-PK either genetically or using a drug inhibiting its function, increases PRRT response in pre-clinical models of NET. The second gene, Beta-Arrestin 2, plays a role in the regulation of the cellular target for PRRT, and we have shown that loss of Beta-Arrestin 2 results in resistance to PRRT. These results and other ongoing studies in our laboratory focussing on these targets will help us to better understand the ways in which cancer cells become resistant to PRRT, and provide insights into what drug combinations might increase the effectiveness of PPRT, with the ultimate goal of improving outcomes for NET patients.