Epigenetic Therapy in Neuroendocrine Tumors

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Background/Significance to NETs

The availability of **Peptide Receptor Radionuclide (Lutathera) Therapy** (PRRT) for neuroendocrine tumors and the possibility of developing peptide drug conjugates targeting the SSTR have encouraged us to develop strategies to increase SSTR expression. Increased SSTR expression has the potential to increase the efficacy of SSTR therapies by increasing available target. For the 175,000 people in the U.S. who are living with this diagnosis, increased expression and increased efficacy of therapies already shown to prolong survival could be of major benefit.

Materials and Methods/Experimental Approach

We have examined a spectrum of neuroendocrine cell lines and have been able to demonstrate induction of the SSTR in a subset of cell lines, but not in all, including cell lines of lung and prostate origin. Specifically, using romidepsin, a histone deacetylase (HDAC) inhibitor approved by the FDA for the therapy of cutaneous and peripheral T-cell lymphoma, we have been able to increase the expression of SSTR2 and SSTR5 in neuroendocrine models. The increases occur quickly at nanomolar concentrations of romidepsin, making the observations clinically relevant. Note this is a strategy that looks to modulate gene expression not to induce cell death as has been the basis of epigenetic agent regulatory approvals to date. Consequently we have looked to induce expression of the SSTR at concentrations that are not or only minimally cytotoxic.

Results & Key Findings

With romidepsin increases have been observed at single digit nanomolar concentrations, begin within 24 hours of drug administration, and increase further with continued exposure up to 96 hours. The increase is sustained after the removal of drug for at least 48 hours. Increased SSTR expression confers increased sensitivity to an SSTR targeted agent but ongoing studies looking at target engagement have been designed to discriminate between greater toxicity from simple additivity of two cytotoxic agents as opposed to greater toxicity from increased SSTR expression and drug delivery with enhanced target engagement.

Conclusion/Next Steps

Our goal is to develop a regimen/strategy that allows us to use epigenetic agents including HDAC inhibitors, DNA methyltransferase (DNMT) inhibitors and Enhancer Of Zeste 2 Polycomb Repressive Complex 2 Subunit (EZH2) inhibitors for brief periods of time to induce a transient increase in surface expression of SSTR2, and in turn increase delivery of radiolabeled pharmaceuticals or peptide drug conjugates targeting the somatostatin receptor.

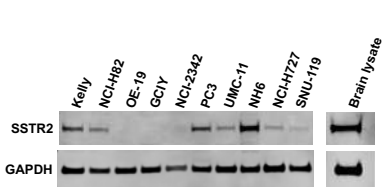


Figure – [Above] Baseline expression of somatostatin receptor 2 (SSTR2) in a diverse group of cell lines.
Neuroblastoma: Kelly, NH6
Neuroendocrine: NCI-H82, NCI-H727, UMC-11
Gastric adenocarcinoma: OE-19. GCIY
Lung adenocarcinoma: NCI-H2342
Neuroendocrine prostate cancer (NEPC): PC3
Ovarian: SNU-119
25 µg protein / lane; SSTR2 (A-8) from Santa Cruz

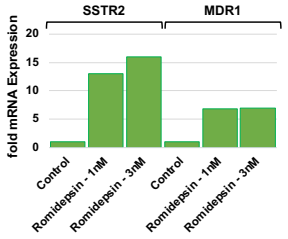


Figure – [Above] Induction of SSTR2 mRNA expression in H727 NET cells by the histone acetylase (HDAC) inhibitors, romidepsin and entinostat. Induction of the drug transporter, MDR1 as a control

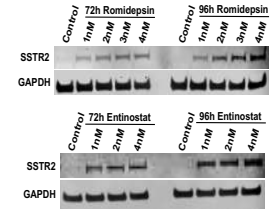
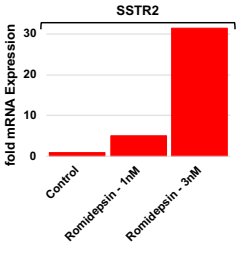


Figure – [Above] Impact of the histone deacetylase (HDAC) inhibitors – romidepsin and entinostat – on the mRNA expression of SSTR-2 in H727 neuroendocrine cancer cells. 25 µg. SSTR2 (A-8) from Santa Cruz.



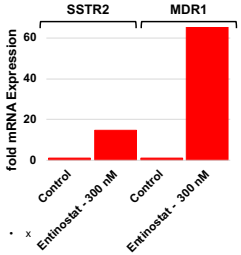


Figure – [Left] Induction of SSTR2 mRNA expression in H82 NET cells by the histone acetylase (HDAC) inhibitors, romidepsin [a class 1 / pan-HDAC inhibitor – administered intravenously] and entinostat [a pure class 1 HDAC inhibitor – administered orally]. In the lower panel induction of the drug transporter, MDR1, known to be induced by HDAC inhibitors, is shown as a control. mRNA levels assesse by PCR.

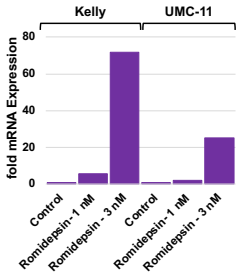


Figure – [Above] Induction of SSTR2 mRNA expression in Kelly, neuroblastoma and UMC-11 NET cells by the histone acetylase inhibitor, romidepsin.

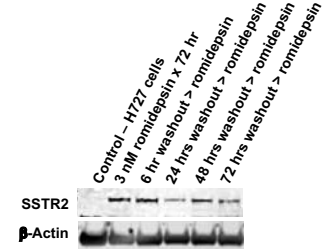


Figure – [Above] Stability of induction of expression of somatostatin receptor with the histone deacetylase (HDAC) inhibitor romidepsin. H727 cells treated for 72 hours with 3 nM romidepsin were rinsed free of romidepsin and SSTR2 expression followed in drug-free medium. 72 hours after being transfereed to romidepsin-free medium expression of SSTR2 was still detectable above control levels

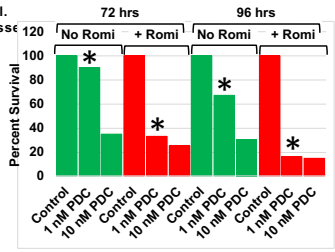


Figure – [Left] Increased toxicity with therapies that exploit the SSTR.
Induction of SSTR2 expression in H82 NET cells resulted in enhanced toxicity of a peptide drug conjugate that carries a cytotoxic payload linked to lanreotide. At both 72 and 96 hours one can see enhanced cell killing in cells with higher levels of SSTR induced by romidepsin (Romi) treatment

CONCLUSIONS:

Our strategy recognizes that:

1. Targeting the SSTR is a safe and effective clinical strategy – FDA and EMA approvals for:
   - Octreotide
   - Lanreotide
   - Lutathera [lutetium Lu^{177} DOTATATE]

   As treatments for NETs
2. Exploiting the SSTR to deliver a radioactive payload is a validated strategy: Lutathera®
3. Delivery of cytotoxic payloads is a validated strategy with FDA and/or EMA approvals for numerous antibody drug conjugates (ADCs). A similar strategy can be envisioned for the delivery of cytotoxic payloads.
4. Strategies that can increase expression of the SSTR can be exploited to augment the delivery of payloads potentially leading to (a) greater efficacy; (b) improved therapeutic window; and (c) the opportunity to offer SSTR-targeting therapies to a greater number of patients

Corresponding Author: Susan E. Bates - email: seb2227@cumc.columbia.edu ←→ COI: None / Funding Acknowledgment: Columbia University ←→ Neuroendocrine Tumor Research Foundation (NETRF) 2020 Symposium