# B-2

## Multiple Layers of Epigenetic Regulation Cooperate to Silence Expression of Somatostatin Receptor Type 2 in Pancreatic Neuroendocrine Tumors

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### BACKGROUND

Pancreatic neuroendocrine tumors (P-NETs) are a rare cancer with increasing incidences worldwide. Low-grade P-NETs are unique in that they express high levels of Somatostatin Receptor Type 2 (SSTR2), which represents a target for both tumor imaging and therapeutics. P-NET grade inversely correlates with SSTR2 tumor staining, and higher tumor grade is associated with poor patient prognosis. Unfortunately, application of SSTR2-targeted treatment options is currently limited in high-grade P-NETs, due to loss of SSTR2 expression. Beyond understudied promoter CpG DNA methylation and nebulous histone deacetylation events, little is known regarding the full scale of actual epigenetic events that conspire to negatively control SSTR2 expression. The goal of our ongoing studies is to obtain a comprehensive understanding, at the molecular level, of the epigenetic events and players which control SSTR2 expression.

### METHODS

Two high-grade P-NET cell lines, BON1 and QGP1 (both with low SSTR2 expression), and one low-grade P-NET cell line, NT-3 (high SSTR2 expression), were employed in our studies. Both small molecule inhibitors/drugs and validated shRNA, targeting various epigenetic enzymes, were utilized in functional assays to determine their potential effects on SSTR2 expression. Western blot analysis was used to gauge potential increased expression of SSTR2, along with changes in global expression levels of selected epigenetic marks. Chromatin immunoprecipitation (ChIP) was used to measure levels of specific histone-based epigenetic marks located on the SSTR2 gene promoter. Bisulfite Next-Generation Sequencing was employed to determine, both qualitatively and quantitatively, levels of CpG methylation in SSTR2 gene regulatory elements.

### RESULTS

We have demonstrated that DNMT3B is the sole DNA methyltransferase responsible for silencing SSTR2 expression in P-NETs. Additionally, we identified Class I HDACs as the main histone deacetylases important for SSTR2 expression regulation. Furthermore, we discovered that Polycomb Repressor Complexes 1 and 2 (PRC-1 and PRC-2) are central to SSTR2 silencing. Removal of activating histone H3K4 methylation marks is an additional mechanism for silencing SSTR2 expression. Finally, we identified the chromatin remodeling enzyme, Lymphoid-Specific Helicase (LSH), likely in tandem with DNMT3B, as a negative regulator of SSTR2 expression in P-NETs.

## CONCLUSIONS

Multiple inhibitory epigenetic mechanisms cooperate to silence expression of SSTR2 in P-NETs. Knowledge gained from our studies will assist in formulation of novel epigenetics-based intervention strategies, to increase expression of SSTR2, for improved imaging and therapeutic treatment of high-grade P-NETs.

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