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Somatostatin Receptor Activation by Agonist-like Antibodies can Reverse miR-200c/ZEB1 Dependent Cell Dedifferentiation and Increase Cell Death in Neuroendocrine Tumor Cells

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Background: Somatostatin receptors (SSTR) are effective therapeutic targets for neuroendocrine tumors. SSTR are comprised of five major subtypes, namely subtypes 1, 2 (A and B), 3, 4 and 5. Previously, we reported that SST signaling is a determinant for the TGF-beta dependent neuroendocrine-mesenchymal transition (NMT) process in BON cells. NMT is similar to the epithelial-mesenchymal transition (EMT) in epithelial cancers, but has the opposite cellular differentiation outcome in response to the TGF-beta treatment. In EMT, the miR-200c has been shown to negatively regulate ZEB1 (an E-cadherin's transcriptional repressor) and keeping EMT invasiveness in check. Here we examined the participation of miR-200c in NMT, and the anti-SSTR antibodies' impact on both NMT and neuroendocrine tumor cell death.

Methods: BON cells were transiently transfected with 40nM XmiR-200c (miR-200c repressor, Oligoengine Inc.) for 30 minutes before adding 10 μg/ml rabbit IgG (negative control), 100nM Octreotide and 10 μg/ml anti-SSTR2,3 or 5 antibody. Next, 48 hours after treatments, cells were collected for Western blot analysis to detect NMT related protein changes. Alternatively, 40 minutes after treatments, we used live cell microscopy to record BON cells' migratory behavior in real time for the next 14 hours. BON and CNDT2.5 cells were treated with octreotide and anti-SSTR antibodies in suspension, cell mortality was determined using Guava viacount, Nexin assay, or MTS proliferation assays.

Results: The XmiR-200c transfected BON cells have elevated ZEB1 protein level, with decreased E-cadherin, and increased Vimentin and Twist mesenchymal markers. The transfected BON cells also displayed an increase in mobility as exhibited under the live cell microcopy. Anti-SSTR antibody treatments reversed BON cells back to the differentiated state even in the presence of XmiR-200c. BON and CNDT2.5 cells both have shown increased cell death upon anti-SSTR antibody treatments.

Conclusions: Similar to EMT, miR-200c and ZEB1 are involved in the cellular dedifferentiation in NMT. Anti-SSTR antibodies are successful in overcoming the effect of XmiR-200c by reversing metastasis marker expressions with decreasing cellular mobility and increasing neuroendocrine tumor cell death.