B4

Specific Glycogen Synthase Kinase-3 Inhibition Suppresses Growth and Reduces the Neuroendocrine Tumor Markers in Neuroblastoma Cells

Muthusamy Kunnimalaiyaan[1]; Yvette M. Carter[1]; Selvi Kunnimalaiyaan[1]; and Herbert Chen[2]

[1]Division of Surgical Oncology, C4763 Translational and Biomedical Research Center, Medical College of Wisconsin Cancer Center, 8701 Watertown Plank Road, Milwaukee, WI 53226
[2]University of Wisconsin Endocrine Surgery Laboratory, Madison, WI and Medical College of Wisconsin, Milwaukee, WI

Background: Neuroblastoma (NB) is a common neuroendocrine tumor (NET) with a high incidence of malignancy and recurrence. NB is very rare in adults but usually present in early childhood. NETs express high levels of achaete-scute complex-like1 (ASCL1) protein and chromogranin A (CgA) peptide, two important NET markers. Despite recent advances, about 60% of patients with high-risk NB will have a recurrence and treatment options for these patients are limited. The glycogen synthase kinase-3 (GSK-3) pathway is a potential therapeutic target, as this pathway has been shown to be crucial in the management of other NETs. However, it is not known which isoform is necessary for the growth inhibition. In this study, we investigate the effects of GSK-3 inhibitor AR-A014418 on the different GSK-3 isoforms in neuroblastoma.

Methods: NGP, SH-5Y-SY and SK-N-AS cells were treated with 0-20μM of AR-A014418. MTT assay was performed to assess growth, and expression of NET markers CgA and ASCL1, GSK-3 isoforms, and apoptotic markers were determined via western blot.

Results: Neuroblastoma cells treated with AR-A014418 had a significant reduction in growth at all doses and time points ($p<0.001$). A reduction in growth was noted in cell lines on day 6, with 10μM (NGP-53% vs. 0% and SH-5Y-SY-38% vs. 0%, $p<0.001$) treatments, compared to control, corresponding with a noticeable reduction in NET marker ASCL1 and CgA expression.

Conclusions: For the first time we have shown that the treatment of neuroblastoma cell lines with AR-A014418 reduced the levels of active phosphorylation of GSK-3α at Tyr279 compared to GSK-3β phosphorylation at Tyr216, and attenuated growth via the maintenance of apoptosis. This study warrants future investigation to elucidate the mechanism(s) by which GSK-3α inhibition down regulates the expression of NET markers and growth of neuroblastoma and other NETs.