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Targeting Glycogen Synthase Kinase-3 as a Therapy for Pediatric Neuroendocrine Tumor

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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. It accounts for approximately 9% of infant cancer, arising once out of 8,000 live births. 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 with reference to NET markers, ASCL1 protein and CgA.

Methods: NGP, SH-5Y-SY and SK-N-AS cells were treated with 0-20μM of AR-A014418. Cell growth was determined by MTT, viable count, and colonogenic assays. Also, non-invasive cellular proliferation assay in real time to measure cell proliferation using IncuCyte Live-Cell Imaging system was carried out in SK-N-AS cells. The levels of NET markers CgA and ASCL1, the expression of GSK-3 isoforms, and apoptotic markers were determined by 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. This was confirmed by real-time imaging and cell viability after treatment. The growth suppression effect by GSK-3 inhibitor is due to apoptosis. Importantly, reduction in GSK-3 activity resulted in attenuation of NET markers ASCL1 and CgA expression.

Conclusions: 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, without change in total GSK-3 and attenuated growth by triggers apoptosis proteins. This study opens new avenue to improve to elucidate the mechanism(s) by which GSK-3α inhibition down regulates the expression of NET markers and growth of neuroblastoma and other NETs.