CgA Depletion Inhibits Neuroendocrine Tumor Cell Growth and Alters Phenotype

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Background: Chromogranin A (CgA) is an acidic soluble protein of the granin family, and overexpressed in NETs. Serum CgA has been widely used as a biomarker for the diagnosis and prognosis of various NETs. However, the biological function of CgA in modulation of NET growth and differentiation has been largely unexplored.

Methods: We employed shRNA knockdown and CRISPR-Cas9 knockout approaches to deplete CgA in human neuroblastoma SHSY-5Y and human pancreatic neuroendocrine tumor (pNET) Bon-1 cells and monitored the actions of CgA depletion on tumor cell growth.

Results: Using short hairpan RNA (shRNA) targeting sequences to CgA Exon 2, we attained potent CgA knockdown as demonstrated by real-time PCR and Western Blotting. Upon CgA knockdown, two changes in the neuroblastoma phenotype were observed. Firstly, tumor cell growth was inhibited by 70% (Day 5), leading to a 1.5 fold reduction in the doubling time of CgA knockdown cells. Secondly, the cell morphology changed from a neuroblastic (N)-type to a so call "substrate-adhesive" (S)-type. Simultaneously, we demonstrated that treatment with metformin alone inhibited neuroblastoma cell proliferation by ~30% in control nonsense SHSY-5Y cells. Addition of metformin to the CgA knockdown SHSY-5Y cells resulted in further inhibition of proliferation by 80%. These findings show a synergetic action of metformin with CgA knockdown to inhibit NET growth. In support of our findings using CgA shRNA, a marked growth inhibition caused by CRISPR-Cas9-mediated CgA knockout was also observed in SHSY-5Y cells concomitant with differentiation toward an S-type. Additionally, we demonstrated that in pNET Bon-1 cells, CgA knockdown also resulted in marked inhibition of tumor cell growth.

Conclusion: Reducing CgA expression in neuroblastoma cells and pNET Bon-1 cells inhibits cell proliferation, promotes cell differentiation and sensitizes the cells to the anti-proliferative actions of metformin. It further suggests CgA may be a promising target for treatment of neuroblastoma and potentially other NETs.

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