TR4: A Potential Therapeutic Target in NETs

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Background: Testicular Nuclear Receptor 4 (TR4) is an orphan nuclear receptor and our recent findings show that it is an important regulator of tumor growth and hormone biosynthesis in pituitary-derived neuroendocrine tumors (NETs). Here we sought to define the broader role of TR4 in the pathogenesis of NETs.

Methods: We employed short hairpin RNA to silence TR4 expression in SHSY-5Y neuroblastoma and PC-12 pheochromocytoma cells and tested actions of TR4 on cell proliferation and differentiation. Simultaneously, we employed a TR4-overexpressing plasmid in gain-of-function studies.

Results: TR4 knockdown in SHSY-5Y cells and PC12 cells inhibited proliferation by 24% and 15% respectively ($p<0.05$). TR4 knockdown in SHSY-5Y cells promoted cell morphological changes from a neuronal (N)-type to a substrate adhesive (S)-type as evidenced by real time PCR analysis showing marked reductions in expression of the N-type cell markers, GAP43 and Synaptophysin, and increased expression of the S-type cell markers, Vimentin, Fibronection and alpha-SMA in the TR4 knockdown cells compared with control. Additionally, whereas all-trans retinoic acid (atRA) treatment promoted differentiation of wild type SHSY-5Y cells, atRA did not inhibit cell proliferation or promote differentiation in the TR4 knockdown cells, further indicating that TR4 knockdown readily promoted terminal differentiation of the neuroblastoma cells toward a fibroblast-like phenotype. In contrast, TR4 overexpression led to increased SHSY-5Y cell proliferation. We measured expression of potential downstream targets of TR4 and observed dramatically reduced Chromogranin A (CgA) mRNA and protein expression in the TR4 knockdown cells. CgA knockdown in the SHSY-5Y cells also led to cell differentiation toward an S-type concomitant with potent inhibition of proliferation.

Conclusion: TR4 blockade inhibits cell proliferation and promotes terminal differentiation in neuroblastoma cells by actions that include modulating CgA expression. These findings indicate a potential role for TR4 in the regulation of NETs pathogenesis, and may pave the way for novel TR4-directed therapies in NETs.

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