Identification of Therapeutic Compounds for Treating Huntington's Disease: High Content Screening of Small Molecules for Modulating N17 Phosphorylation of Mutant Huntingtin

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Huntington's disease (HD) is an incurable neurodegenerative disorder characterized by physical, behavioral, and cognitive impairments. It is caused by trinucleotide repeat expansions in the huntingtin (Htt) gene. The first 17 sequence of amino acids, known as the N17, is a crucial moderator of huntingtin protein localization and aggregation. Recent studies have shown that mutant huntingtin exhibits hypophosphorylation of N17 in contrast to the normal protein. Currently, traditional methods are limited to HD models relying on over-expression of Htt gene fragments which often leads to skewed and irrelevant results. Using a novel yet inexpensive approach via high-content screening focused on the endogenous huntingtin, this project identified biologically applicable therapeutic compounds that can restore protein function. 60 FDA approved small molecules were applied on striatal neurons expressing mutant Htt seeded to a 96 well plate. After cells were stained with a primary anti-N17-phospho antibody and Hoechst dye, they were imaged by fluorescence microscopy. The MDS output based on phenotypic analysis indicated that compounds axitinib and pemetrexed show potential for increasing the longevity of striatal cells by modulating N17 phosphorylation state of mutant huntingtin. Paradoxically, pazopanib HCl was identified among others as having a deleterious effect on striatal cells and increased toxicity by causing stress-induced N17 phosphorylation. While localization of phospho-huntingtin in the nucleus was observed in the unhealthy cells, the healthy cells displayed an antibody dense ring-like structure around the nucleus. Axitinib was further validated through Western Blot analysis. Findings are promising for development of the first pharmacological treatment for HD.