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Influence of CYP46A1 protein levels in the brain of Ki150 mouse model in the context of the role of cholesterol turnover in the pathogenesis of spinocerebellar ataxia type 3.

Disorders of cholesterol metabolism in the brain are associated with many neurodegenerative diseases, such as Alzheimer's, Parkinson's, Huntington's diseases, spinocerebellar ataxia type 3 (SCA3), and neurological diseases such as schizophrenia, epilepsy, and autism. SCA3 is a neurodegenerative disease, currently incurable. One of the main brain structures degenerated in SCA3 is the cerebellum, where the cerebellar Purkinje neurons (PCs) play an essential function in cholesterol metabolism and are the primary source of cholesterol 24-hydroxylase (CYP46A1). The enzyme affects the cholesterol level in the brain by controlling the excretion of this compound across the blood-brain barrier. CYP46A1 is also a vital activator of the mevalonate pathway, whose downstream products affect signaling pathways regulating important cellular and molecular processes often associated with neurodegeneration. Therefore, modulation of CYP46A1 levels may be the basis for effective SCA3 therapy delivered with AAV carriers already being used in gene therapies.

Successful gene therapy in nervous system diseases delivered with AAVs depends on achieving satisfactory biodistribution, which in the case of SCA3, should be focused on PCs. Based on experiments conducted in the dissertation, the properties of AAVrh10 and AAV-PHP.eB viral vectors were discovered. The AAVrh10 and AAV-PHP.eB viral vectors can transport genetic load in anterograde and retrograde directions. Using the cerebellar cell projection network and the direct injection technique, we achieved transduction of a wide pool of cerebellar cell populations with a reproducible transduction pattern. We have developed a method for selective and efficient direct stereotactic injections using the aforementioned viral vector serotypes, particularly for the transduction of cerebellar PCs by administration to the deep cerebellar nuclei (DCN).

Alteration of CYP46A1 can change cholesterol levels in the brain and thus affect the course and disease phenotype severity related to cholesterol metabolism. We have shown that the levels CYP46A1 enzyme are reduced in the cerebellum of SCA3 Ki150 transgenic mice that exhibit motor changes similar to those in SCA3 patients. We hypothesized that the changes in patients and the phenotypic changes in SCA3 Ki150 mice might be related to impaired cerebellar cholesterol metabolism.

Administration of shRNA reagents that modulated CYP46A1 levels to the WT and SCA3 Ki150 models resulted in the inhibition of *CYP46A1* expression. Tests showed improved motor performance in mice that received shRNAs that decreased *CYP46A1* expression compared to corresponding control groups. We present one of the first results linking the reduction of CYP46A1 levels in the murine with improved phenotype in a mouse model of WT and SCA3 Ki150. Reducing *CYP46A1* gene expression, and consequently a decrease in CYP46A1 protein levels, may improve Purkinje neuronal function by promoting neuronal survival as a result of the possible activation of compensatory mechanisms. We postulate the CYP46A1 as SCA3 therapeutic target, and we underline the necessity of a broader understanding of the role of *CYP46A1* expression level and the mechanism mediating disease amelioration in SCA3.