Efficacy of shRNA-mediated non-allele-specific partial suppression of huntingtin in patient-derived iPS cells

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Our research seeks to develop a potential therapy for Huntington's disease. An adeno-associated viral vector encoding for a short hairpin RNA (shRNA) that targets both human and rhesus huntingtin (Htt) was delivered bilaterally to the caudate and putamen of rhesus monkeys. This construct achieved sustained expression of the shRNA transcript and reduced levels of Htt mRNA by 30% and wildtype protein by 45%. After 6 months, no adverse reactions or evidence of neuropathology were detected (Grondin, et al., 2012). The objective of our research is to determine if that same non-allele-specific agent will enhance the survival of neuron-like cells derived from induced pluripotent cells provided by heterozygous HD patients (i-neurons). Pre-clinical studies of the efficacy of shRNA and other Htt-lowering agents in transgenic mouse models are valuable. However, they often have inherent limitations due to unequal baseline levels of expression of the normal and expanded transgenic alleles, and unknown differences in the contextual influence of the animal's transcriptome and that of a human HD patient. Therefore, use of a cellular model of HD to investigate the safety and efficacy of lowering Htt levels may provide key information that complement the findings from animal models.

We are developing two plasmids containing a green fluorescent protein expression cassette and the coding sequence for the active or control shRNA noted above. One or the other of these plasmids, with a MAP2-mApple plasmid as a marker of mature neuronal differentiation, will be co-transfected into i-neurons derived from patients with 60 or 180 CAG repeats or from a control volunteer (33 CAGs). Transfected neurons will be tracked over time by robotic microscopy to determine if the shRNA rescues indicators of cellular dysfunction, including the risk of cell death and neurite retractions. If non-allele-specific suppression in cells heterozygous for normal and pathogenic Htt CAG repeat lengths is safe and effective, we expect a treatment-dependent improvement in the status and survival of HD i-neurons. Conversely, if the status or survival of HD i-neurons is worsened, it will suggest that allele-specific methods for lowering mutant Htt should be given priority among Htt-lowering therapy development efforts, despite the greater regulatory costs that the development of such therapies will entail.

Support: Hereditary Disease Foundation, CGTA Research Group