SMA THERAPY: POSTER PRESENTATIONS

P3.10
Intramuscular AAV9 administration enables transgene delivery to motor neurons in the whole spinal cord: Therapeutic application in a SMA mouse model
S. Benkhelifa-Ziyyat, A. Besse, S. Duqué, R. Carcenac, T. Maraïs, S. Astord, M. Roda, M. Barrats
Institut de Myologie, Biothérapie des Maladies Neuromusculaires, UMR S974, INSERM U 974, CNRS UMR 7215, Université Pierre et Mar, Institut-Myologie, Paris, France

We recently showed that a single intravenous injection of an optimized SMN-encoding scAAV9 (scAAV9-SMNopti) rescued a Spinal Muscular Atrophy (SMA) mouse model. Intramuscular (IM) injection of AAV1–AAV8 has been reported to mediate a moderate spinal cord transduction through axonal retrograde transport. Given the remarkable effectiveness of scAAV9 for motor neurons (MNs) gene transfer, we analyzed whether IM injection of this vector was efficacious to target MNs throughout the entire spinal cord. A single injection of scAAV9-GFP was performed into the right gastrocnemius of adult mice (n = 16) and GFP expression was analyzed 4 week later by immunofluorescence analysis on transversal spinal cord sections. The analysis of GFP expression revealed an efficient transduction of MNs throughout the lumbar spinal cord. Unexpectedly, cells in the thoracic and cervical spinal cord (which do not innervate the gastrocnemius) were also highly transduced, with the highest expression observed in the cervical segments. We therefore investigated the interest of this approach for SMA gene therapy in a SMNdelta7 mouse model. Neonatal muscle delivery of increasing doses of scAAV9-SMNopti in this model (n = 32), allowed restoration of survival in 100% of the treated mice. At the highest dose, the treatment increased life expectancy from 163 to over 227 days, with to date a mean survival of 188 ± 20 days versus 13.7 ± 0.6 days for untreated SMA mice. The intramuscular treatment also preserved motor activity, consistent with a protective effect on motor units. This study demonstrates, for the first time, that a single intramuscular scAAV9 injection into the hind limb of adult mice mediated MNs transduction in the whole spinal cord, and that this strategy was efficient to rescue a mouse model of severe SMA.

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P3.11
Antisense oligomer mediated retention of SMN2 intron and exon 7 leads to a novel SMN transcript and increased functional SMN protein in Spinal Muscular Atrophy fibroblasts
C. Mitrpan, L. Price, R.D. Johnsen, A.H. Burghes, S. Fletcher, S.D. Wilton
University of Western Australia, Centre for Neuromuscular and Neurological Disorders, Perth, Australia; Ohio State University, Department of Molecular and Cellular Biochemistry, Columbus, United States

Spinal Muscular Atrophy (SMA) is the leading genetic cause of death in children under the age of 2 years. The overall incidence of SMA is in the order of 1 in 10,000 live births with a carrier frequency of 1/35. SMA is caused most commonly by deletion of the Survival Motor Neuron 1 gene (SMN1). Although the human genome also contains an extra centromeric copy of SMN1, the SMN2 gene cannot adequately compensate for SMN1 loss unless present at high copy number, since the majority of SMN2 transcripts are preferentially transcribed with the SMN transcript missing exon 7 (SMNδ7), resulting in the loss of functional SMN protein. The SMNδ7 mRNA is translated into a truncated protein, which is unstable and cannot self-associate, and therefore leads to unstable SMN protein. The use of antisense oligomers (AOs) to mask splice motifs can interfere with splice site recognition by the spliceosome; hence allowing alteration of the splicing pattern of the target gene. Others have been focusing on masking the splice-silencing motifs located in either intron 6 or intron 7. In contrast, our work using AOs targeted to the acceptor site of exon 8 has proven to induce readily detectable exon and intron 7 containing transcript (SMN+int7). 48 h after transfection in fibroblasts from a type I SMA patient. A panel of AOs were designed to refine the location of the potential target sequences in SMN exon 8 to induce the SMN+int7 transcript. Furthermore, western blot analysis has demonstrated increased amounts of SMN protein in SMA fibroblasts treated with these AOs. Substantially increased frequency of Gems in the treated fibroblasts demonstrated the functionality of the induced SMN protein. Additionally, this splice intervention has considerable therapeutic potential for the treatment of patients with SMA.

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P3.12
Small molecule compounds that correct alternative splicing of the SMN2 gene and restore SMN protein expression and function
PTC Therapeutics, South Plainfield, United States; SMA Foundation, New York, United States

Spinal muscular atrophy is caused by the reduced expression of the survival motor neuron (SMN) protein due to the loss of functional SMN1 gene and alternative splicing of exon 7 in the nearly identical SMN2 gene which predominantly generates truncated and unstable SMNδ7 protein. PTC Therapeutics, Inc. is pursuing innovative drug discovery strategies aimed at restoring the expression of the SMN protein at the post-transcriptional level. Panels of cell based assays and animal models have been established and optimized to assess the effects of compounds on SMN mRNA and protein expression and function. Orally bioavailable small molecules have been identified that cross the blood–brain barrier, increase the inclusion of exon 7 into the SMN mRNA and increase the levels of SMN protein in cultured cells from SMA patients. In mouse models of severe and mild SMA, these compounds increase full length SMN mRNA and protein in various tissues. In addition, in the severe SMA mouse model the compounds increase median survival by more than 300% and result in improved motor function. Lead compounds from this program are undergoing further medicinal chemistry optimization of biological, pharmacological and pharmaceutical properties with the ultimate goal of identifying molecules for preclinical and clinical development.

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P3.13
Phenolic compounds and their effect on full length and delta7 SMN2 transcripts
D. Dayangac-Erden, G. Bora-Tatar, S. Dalkara, H. Erdem-Yurter
Hacettepe University Faculty of Medicine, Medical Biology, Ankara, Turkey; Hacettepe University Faculty of Pharmacy, Pharmaceutical Chemistry, Ankara, Turkey

Phenolic compounds gained attention for treatment of some neurodegenerative diseases. SMA which is an autosomal recessive childhood onset disease is characterized by muscle weakness and degeneration of alpha
Spinal muscular atrophy (SMA) is among the most common genetic neurological diseases that cause infant mortality. The possibility of reprogramming mature somatic cells to generate induced pluripotent stem cells (iPSCs) has enabled the derivation of disease-specific pluripotent cells (iPSCs). However, derivation of iPSCs often requires vectors that integrate into the genome, limiting both its research and clinical applications. We developed iPSCs from fibroblasts from a patient with type I SMA and his unaffected father using a non-viral method. Cells were transfected with oriP/EBNA1 vectors encoding six reprogramming factors. We generated SMA and WT iPSCs from exogenous sequences. The cells had morphologic and transcriptional similarities with ES (on microarray analysis) and differentiated into all three germ layers in vitro and in vivo. iPSCs were differentiated into post-mitotic motoneurons that express motoneuron-specific transcription factors such as HB9 and Isl1 as well as ChAT. Motor Neurons derived from SMA-iPSCs had significant differences in comparison to WT motoneurons, including reductions in cell number, cell size, and axon length. Next, we performed in vivo analysis evaluating whether and how iPSC-derived motoneurons integrated into the SMA spinal cord. We detected human-derived motoneurons, which presented motoneuronal phenotype and coexpressed HB9 and ChAT, within the ventral horns of all transplanted animals. Quantification data demonstrated that SMA motoneurons presented a reduced number of engrafted cells compared with WT. Motoneuron (WT and SMA) transplantation extends lifespan (>50%) and ameliorates the phenotype of SMA mice. Our data provide evidence that it may be possible to generate patient-specific iPSCs and motoneurons free of exogenous elements with potential value for research and clinical applications.

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