Proteomic evaluation of Pip6a-PMO treatment for Myotonic Dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy in adults. DM1 is caused by the pathological microsatellite (CTG) repeat expansion in the 3'untranslated region of the *DMPK* gene. Mutant RNAs containing the repeat CUG expansions are retained in the nucleus as foci and sequester proteins required for the regulation of mRNA splicing and translation. These perturbations result in a multisystemic disorder characterised by myotonia, progressive muscle weakness, cardiac arrhythmias, cataracts and impaired endocrine and nervous system function. To date there is no cure for DM1. Antisense oligonucleotides (ASOs) are a promising genetic therapy for RNA gain-of function diseases like DM1. A leading strategy for an enhanced delivery system is modulation of ASO chemistry though peptide conjugation. This allows for tissue-specific delivery while directly targeting CUG repeat expansions that can interfere with abnormal sequestration and binding of RNA-binding proteins like MBNL1.

This study investigates the systemic delivery ASO conjugated to an arginine-rich cell-penetrating peptide, Pip6a-CAG_7-PMO, in the HSA^{LR} mouse model of DM1. Therapeutic restoration was successfully achieved as seen by reduced foci, redistribution of MBNL1, normalisation of altered splicing patterns and reduced myotonia. To assess deeper treatment efficacy at the protein expression level, global mass spectrometry based proteomic analyses of the HSA^{LR} mouse muscle was employed. Treatment of Pip6a-PMO induced a positive widespread shift in protein expression levels towards a healthy phenotype. Comparative analysis between datasets showed that protein ratios were strongly correlated and differentially affected cellular protein pathways have been identified. This proteomics experiment provides fundamental new insights into protein expression and regulation of myotonic muscle. This research provides a comprehensive insight for differential protein expression in myotonic muscle pre-treatment and restoration of protein homeostasis post Pip6a-PMO treatment. Pip6a-PMO demonstrates encouraging therapeutic potential for DM1.

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