Poster number 46

Enhancing Antisense Oligonucleotide Activity in Myotonic Dystrophy Type 1 by Genetic and Chemical Modulation

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BACKGROUND & THERAPEUTIC RATIONALE

Myotonic dystrophy type 1 (DM1) is an RNA gain-of-function disorder caused by expanded CTG repeats in the DMPK gene[1]. Mutant DMPK transcripts accumulate as nuclear RNA foci and sequester Muscleblind-like (MBNL) proteins, leading to widespread spliceopathy and multisystemic disease manifestations[2]. In addition to toxic RNA-mediated MBNL sequestration, dysregulation of specific microRNAs further contributes to DM1 pathogenesis[3]. In particular, miR-23b negatively regulates MBNL1 expression, while anti-miR-23b therapeutic strategies have demonstrated restoration of MBNL activity and improvement of DM1-associated phenotypes in preclinical models[3]. Antisense oligonucleotide (ASO)-based therapeutic strategies are currently under development for DM1, including RNase H-mediated degradation of toxic DMPK transcripts, steric-blocking approaches and modulation of pathogenic RNA-protein interactions[4]. Multiple ASO-based therapeutic strategies targeting toxic DMPK RNA are currently advancing through preclinical and clinical development (see **Poster 84**: "Therapeutic Innovation, Clinical Trial Acceleration, and Lifestyle Interventions in Myotonic Dystrophy Type 1: A Systematic Review"). Despite promising therapeutic potential, productive intracellular delivery remains a major limitation for oligonucleotide therapeutics. Following endocytic uptake, most ASOs remain trapped within endosomal compartments, severely limiting productive nuclear delivery and therapeutic efficacy[5]. Current approaches to enhance endosomal escape include membrane-disrupting agents, lipid-based carriers, cell-penetrating peptides and modulation of intracellular trafficking pathways[5,6].

Endosomal escape remains a major unresolved barrier in oligonucleotide therapeutics.

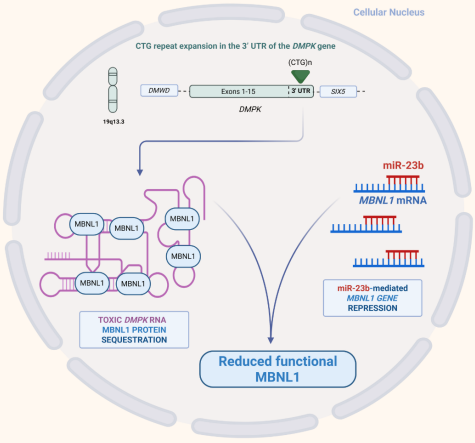

Figure 1. Molecular mechanisms underlying DM1 pathogenesis and therapeutic targeting strategies[1,2,3]. Expanded CTG repeats in the 3' UTR of the DMPK gene generate toxic RNA transcripts that accumulate as nuclear RNA foci and sequester MBNL proteins, leading to reduced functional MBNL1 activity and widespread spliceopathy. In parallel, miR-23b-mediated repression of MBNL1 transcripts further contributes to MBNL loss-of-function in DM1[3]. Current therapeutic approaches aim to reduce toxic DMPK RNA burden and restore functional MBNL activity.

PROJECT HYPOTHESIS

We hypothesize that combined chemical and genetic modulation of intracellular trafficking pathways may enhance PRODUCTIVE ENDOSOMAL ESCAPE and improve therapeutic ASO activity in DM1[4,5].

PROJECT OBJECTIVES

1. ***Identification of non-cytotoxic modulators enhancing productive ASO escape***
2. ***Development of combined chemical and genetic enhancement strategies***
3. ***Improved understanding of intracellular trafficking mechanisms controlling ASO delivery***
4. ***Potential applicability across multiple oligonucleotide-based therapeutic platforms***

INTRACELLULAR TRAFFICKING AND ENDOSOMAL ESCAPE OF ASOs

Although ASOs are efficiently internalized through endocytic pathways, less than 2% of intracellular oligonucleotides are estimated to achieve productive cytosolic or nuclear delivery following endosomal uptake[6]. Most internalized ASOs remain sequestered within endosomal and lysosomal compartments or undergo recycling and degradation, severely limiting therapeutic efficacy. Targeting intracellular trafficking and endosomal dynamics therefore represents a promising strategy to enhance productive ASO delivery.

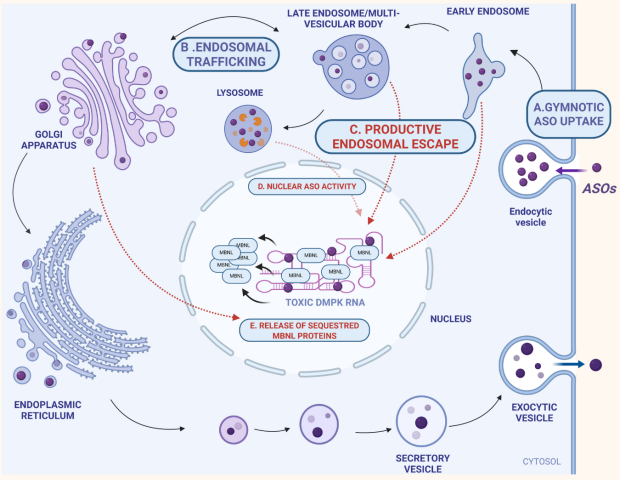

Figure 2. Schematic overview of intracellular trafficking pathways regulating productive ASO delivery in DM1[4,5,6]. **A) GYMNOTIC ASO UPTAKE** mediates cellular internalization through endocytic pathways. **B) ENDOSOMAL TRAFFICKING** through multiple intracellular vesicular compartments, including early endosomes, late endosomes/multivesicular bodies (MVBs), lysosomes, Golgi-associated pathways and secretory vesicles. **C)** PRODUCTIVE ENDOSOMAL ESCAPE may occur from multiple intracellular trafficking compartments and represents a major limiting step for therapeutic ASO activity. **D)** Productive nuclear delivery enables **ASO-MEDIATED STERIC BLOCKADE OF TOXIC DMPK RNA TRANSCRIPTS.** **E)** Reduction of toxic RNA burden promotes release and **FUNCTIONAL RESTORATION OF SEQUESTRED MBNL PROTEINS.**

TRANSLATIONAL PIPELINE

STEP 1-2: Chemical Screening and Functional ASO Evaluation

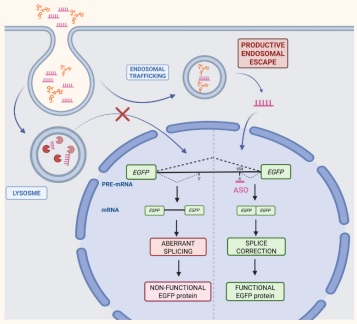

Figure 3. Schematic overview of the HeLa IVS2-654 splice-switching reporter system used for primary ASO activity screening[7,8]. Following gymnotic uptake, intracellular ASOs undergo **ENDOSOMAL TRAFFICKING** and **PRODUCTIVE ENDOSOMAL ESCAPE** before reaching the nucleus. The IVS2-654 reporter contains an aberrant intronic sequence interrupting the *EGFP* pre-mRNA transcript. In the absence of ASO activity, activation of aberrant splice sites generates mis-spliced transcripts and non-functional EGFP protein. ASOs remaining trapped within lysosomes or non-productive intracellular vesicular compartments fail to reach the nucleus and therefore do not restore correct splicing. Splice-switching ASOs bind the aberrant splice site, restore correct pre-mRNA processing and promote functional EGFP protein expression. Candidate chemical modulators are co-administered with ASOs to identify compounds capable of enhancing productive intracellular ASO delivery and splice-correction efficiency.

STEP 3: Validation in Immortalized Myoblasts

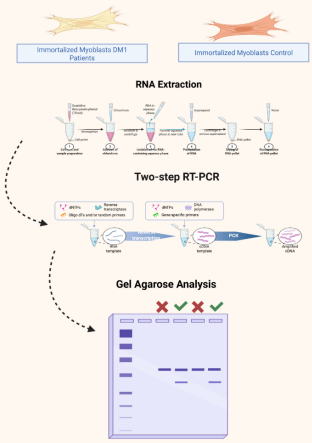

Figure 4. Selected candidate compounds identified during primary screening, together with alternative ASO conjugation strategies, are evaluated in immortalized myoblast splicing reporter systems. Functional ASO activity is assessed through RNA extraction, two-step RT-PCR and agarose gel analysis to quantify **exon inclusion/exclusion patterns and splice correction efficiency** in a biologically relevant muscular cellular context[8].
Semi-quantitative RT-PCR and agarose gel electrophoresis are used to evaluate ASO-induced splice correction. Separation of the upper and lower bands reflects exon exclusion events and successful ASO-mediated splice-switching activity, whereas the presence of a single band indicates aberrant splicing and absence of detectable splice-switching activity.

STEP 4 : Genetic Modulation of Intracellular Trafficking Pathways

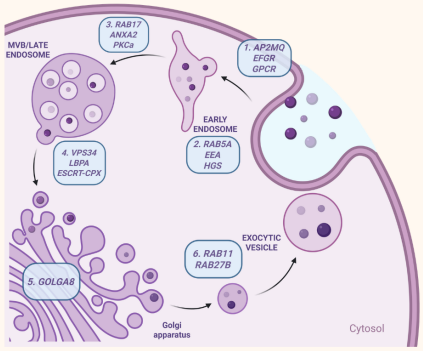

Figure 5. Candidate intracellular trafficking regulators involved in productive ASO delivery are genetically modulated in immortalized myoblast systems to identify pathways controlling intracellular ASO trafficking and endosomal escape[4,5,9].
1. Endocytic uptake regulators: *AP2M1, EGFR, STAB2*
2. Early endosome regulators: *RAB5A, EEA1, HGS*
3. Late endosome/MVB regulators: *RAB7A, VPS4, LBPA*
4. Lysosomal trafficking regulators: *LAMP1, LAMP2*
5. Golgi-associated trafficking regulators: *GOLGA8*
6. *Recycling and vesicular trafficking regulators:* *RAB11A, RAB27B.*

Genetic perturbation of these trafficking nodes aims to identify pathways capable of enhancing productive nuclear ASO delivery.

STEP 5 — Combined Chemical and Genetic Modulation Strategy

Selected chemical modulators are combined with candidate trafficking gene perturbations to evaluate synergistic strategies capable of reducing non-productive endosomal entrapment and enhancing productive nuclear ASO activity.

STEP 6 — Translational Validation in DM1 Models

The most promising combined modulation strategies are evaluated in translational DM1 in vivo models to assess therapeutic ASO activity, systemic biodistribution and safety profiles. Molecular and functional analyses include tissue-specific ASO distribution together with blood- and urine-based toxicity assessment.

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