Reg. No: 2020/37/B/NZ5/01263; Principal Investigator: dr inż. Ewa Agnieszka Stępniak-Konieczna

PROJECT TITLE:

Therapeutic activation and signaling determinants of the autoregulatory *MBNL1* expression in myotonic dystrophy.

INTRODUCTION

The sequestration and functional depletion of *Muscleblind-like* (MBNL) proteins by transcripts derived from the mutant *DMPK* allele harboring CUG repeat expansion (CUG^{exp}) is the central point of the pathomechanism underlying myotonic dystrophy (DM). The sequestration significantly impairs MBNL function in the alternative splicing regulation of many target primary mRNA molecules (pre-mRNA), leading to characteristic disease symptoms including myotonia, skeletal and cardiac muscle myopathy, as well as cataracts. Despite extensive research, DM is still incurable and patients' treatment is limited to alleviating clinical symptoms and providing supportive care. Importantly, overexpression of exogenous *MBNL1*, the most prominently expressed member of the MBNL family, has the potential to counteract CUG^{exp}-induced toxicity and ameliorate DM pathology. Despite therapeutic relevance, however, the knowledge on the transcriptional regulation of *MBNL* genes and associated signaling pathways is extremely limited. **Our recent work revealed that all MBNL family proteins can precisely regulate MBNL1 protein levels via so-called e1-loop, a natural autoregulatory feedback loop mechanism based on the alternative splicing of the first coding exon (e1) of *MBNL1*. The e1 loop is an important homeostatic mechanism fine-tuning MBNL1 protein pool according to cellular demands and presumably plays pivotal role in delaying the onset of DM. Theoretically, activation of the e1 loop could be used to fight DM by achieving therapeutic and at the same time physiological level of the self-regulating MBNL1 protein.** Importantly, experimental data indicate the MBNL1 may be a stress sensor adjusting its own expression via the e1 loop in response to various stress triggers to prevent cellular damage. Moreover, involvement of MBNL1 in impaired cellular response to stress stimuli has been identified as a key element of DM pathomechanism. **Therefore, it seems pivotal to identify the cellular signaling pathways and transcription factors affecting the expression of the *MBNL1* gene, particularly in the context of DM, cellular stress and the autoregulatory e1 loop.**

AIMS AND SIGNIFICANCE OF THE PROJECT

AIM 1) We will use cellular and murine DM models to **design and test a novel therapeutic strategy against DM based on activation and upregulation of the *MBNL1* gene expression, to generate protein pool large enough to saturate toxic** CUG^{exp} **expansions, as well as bind its pre-mRNA targets to restore their proper alternative splicing.** Molecular methods that we will use for this purpose will allow us to compare therapeutic impacts of **the autoregulatory *MBNL1* expression based on the e1 splicing loop that determines optimal physiological protein levels – an idea not tested so far in DM therapy** - vs unopposed upregulation of highly functional MBNL1 which may be toxic in the longer perspective.

AIM 2) To gain insights into signaling molecules that could be used in DM therapy, especially those determining *MBNL1* autoregulation via e1 loop, we will characterize cell signaling pathways and transcription factors stimulating *MBNL1* expression. We will use a variety of biochemical, molecular and bioinformatics techniques as well as distinct cellular models that will allow studying the effect downstream of MBNL1 in crucial cell fate decisions like proliferation, migration and apoptosis as well as associated signaling pathways.

AIM 3) We will generate a series of simplified molecular tools for studying potential factors affecting the efficiency of e1 splicing, as well as assessing the interaction between e1 skipping and its circularization upon pre-mRNA excision (circRNA). Because circRNAs represent an important level of protein regulation by binding excess MBNL, it is crucial to expose the interaction between these two processes, especially in the context of DM, where the level of functional MBNL determines symptoms severity.

Altogether, data obtained within this project will be a valuable aspect in understanding the regulation of *MBNL1* gene expression, DM pathomechanism as well as revealing new signaling molecules that could be potentially targeted in future therapeutic strategies against this disease.