Neuromuscular Research

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Translational Research in Neuromuscular Diseases

Our Neuromuscular Research Laboratory is part of the Clinic of Neurology and the Department of Biomedicine and focuses on the elucidation of pathophysiological mechanisms involved in neuromuscular diseases and on the development of therapeutic strategies.

Myotonic dystrophy type I (DM1) is a disabling neuromuscular disease with no causal treatment available. It is the most prevalent muscular dystrophy in adults, affecting about 1 in 10'000 individuals. This disease is caused by expanded CTG trinucleotide repeats in the 3' UTR of the dystrophia myotonica-protein kinase gene (DMPK). On the RNA level, expanded (CUG)n repeats form hairpin structures that sequester splicing-factors, such as muscleblind-like 1 (MBNL1). Lack of available MBNL1 leads to mis-regulated alternative splicing of many target pre-mRNAs, causing multisystemic involvement in DM1. In an effort to identify small molecules that liberate sequestered MBNL1 from (CUG)n RNA, we developed a pathomechanism-based screening cascade including biochemical, cellular and animal model assays which allow for high throughput screening of small molecular weight compounds. Identified hits may provide pharmacophores for further medicinal chemistry optimization.

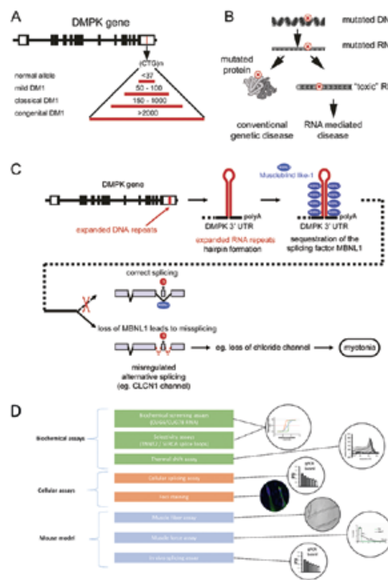

Fig. 1: Pathophysiology of DM1 and screening cascade to identify small molecular weight compounds. A) The molecular basis of DM1 is an expansion of an unstable repeat sequence in the noncoding part of the DMPK gene. Severity of disease is correlated with the size of the repeat expansion. **B)** In DM1, the mutation is located in a noncoding region and does not alter the protein sequence, but leads to toxic RNA. **C)** The sequestration of the alternative splicing factor MBNL1 by toxic RNA leads to altered splicing of target pre-mRNAs like CLCN1, encoding muscle-specific chloride channel (ClC-1). This mis-splicing leads to *ClC-1* deficiency and to myotonia. **D)** We established a pathophysiology based screening cascade including biochemical, cellular and animal model assays to identify small molecular weight compounds able to disrupt the interaction between MBNL1 and the toxic RNA, and to restore splicing and function.

Facio-scapulo-humeral muscular dystrophy (FSHD) is the second most common muscular dystrophy in adults, affecting about 1:20'000 persons. An epigenetic aberration leads to the ectopic expression of the transcription factor Double Homeobox protein 4 (DUX4) in skeletal muscle and other tissues, which leads to muscle cell degeneration and muscular dystrophy, sensorineural hearing loss and retinal teleangiectasias. Expression of DUX4 variants are also involved in certain cancers including acute lymphoblastic leukemia. An attractive therapeutic approach would be the interference with aberrantly expressed DUX4.
By applying Systematic Evolution of Ligands by Exponential Enrichment (SELEX) and fluorescence-based biochemical assays we were able to generate a DNA aptamer with high affinity towards DUX4. In a collaborative effort we co-crystallized DUX4 together with the identified oligonucleotide enabling us to explain the affin-

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ity boost caused by certain bulge loops. We plan to use this oligonucleotide as a tool to further study DUX4-DNA interactions and to develop treatment strategies for FSHD and other DUX4-mediated diseases.

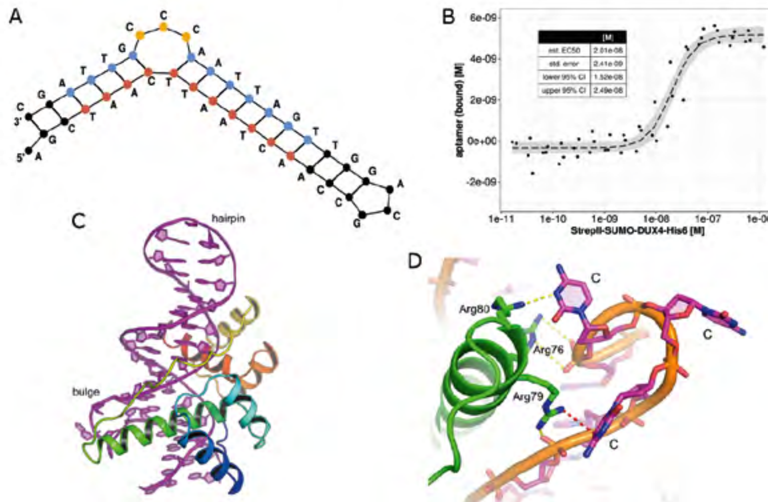

Fig. 2: Generation of a high affinity aptamer against DUX4. A) Predicted secondary structure of the high affinity oligonucleotide. DUX4 binding motif is highlighted in red (forward motif) and blue (reverse motif). Affinity bulge loop is highlighted in yellow. **B)** KD value for DUX4–oligonucleotide binding, determined by fluorescence polarization assay. Data are shown with 95 % confidence band in gray. Fitting results are displayed in the table at the upper left corner. **C)** Crystal structure of DUX4 double homeodomain bound to the DNA oligonucleotide containing a trinucleotide (-CCC-) bulge and a GCA hairpin loop. DNA is shown in magenta. DUX4 is colored in a gradient of blue to red from the N- to C-terminus, respectively. **D)** A close-up view showing the DUX4 interactions with the CCC bulge loop. The guanidinium group of Arg79 stacks onto the first C base of the bulge (the van der Waals or cation-π-contact is indicated by a red dotted line). Arg80 forms a salt bridge with the third C base and Arg76 is hydrogen-bonded to a DNA backbone phosphate at the 3'-end of the bulge (yellow dotted lines)

An additional line of research in our laboratory is dedicated to the identification of mechanisms underlying disruption of proteostasis as a cause for muscle diseases. To this end, we initiated experimental strategies to identify molecular networks that specifically control protein synthesis and degradation in human myofibers under physiological conditions and upon atrophic stress. Our strategy integrates the genome-wide measurements of a.) transcript levels (RNA-Seq), b.) translation levels (sequencing of ribosome foot prints) and c.) protein levels (proteomics) from genetically (Crispr/Cas9) modified human immortalized myoblasts. The ribosome foot-printing method, which we developed together with the laboratory of Prof. M. Zavolan, provides us with estimates of protein synthesis rates of individual RNA molecules, thus allowing us to further uncover the regulatory factors of translation for each gene under normal and diseased conditions. In parallel, we developed in situ proximity-dependent labelling assays (BioID) to map the direct and indirect interactome of the two main proteins we have already identified in skeletal muscle atrophy, MAFbx and EIF3F (Fig. 3).

Connection to Clinical Practice

Michael Sinnreich
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Interdisciplinary Neuromuscular Clinic

At our interdisciplinary Neuromuscular Clinic we care for patients affected by a broad range of neuromuscular diseases. In collaboration with our colleagues from pathology, genetics, plastic surgery, pulmonary medicine, rehabilitation, ergo-, physio- and speech therapy as well as social services, we provide clinical and electrophysiological evaluation, perform muscle and nerve biopsies with histopathological and biochemical workup, genetic workup and counseling, rehabilitation, ergo-/physio- and speech therapy as well as assistance in social matters. Novel clinical observations are being worked up scientifically and form the basis for translational research projects.

Selected Publications

Ham DJ, Börsch A, Lin S, Thürkauf M, Weihrauch M, Reinhard JR, Delezie J, Battilana F, Wang X, Kaiser MS, Guridi M, Sinnreich M, Rich MM, Mittal N, Tintignac LA, Handschin C, Zavolan M, Rüegg MA. The neuromuscular junction is a focal point of mTORC1 signaling in sarcopenia. Nat Commun. 2020 Sep 9;11(1).

Klingler C, Ashley J, Shi K, Stiefvater A, Kyba M, Sinnreich M, Aihara H, Kinter J. (2020). DNA aptamers against the DUX4 protein reveal novel therapeutic implications for FSHD. FASEB J. 2020 Mar;34(3):4573–4590.

Ham AS, Chojnowska K, Tintignac LA, Lin S, Schmidt A, Ham DJ, Sinnreich M, Rüegg MA. mTORC1 signalling is not essential for the maintenance of muscle mass and function in adult sedentary mice. J Cachexia Sarcopenia Muscle. 2020 Feb;11(1):259–273.

Castets P, Rion N, Théodore M, Falcetta D, Lin S, Reischl M, Wild F, Guérard L, Eickhorst C, Brockhoff M, Guridi M, Ibebunjo C, Cruz J, Sinnreich M, Rudolf R, Glass DJ, Rüegg MA. mTORC1 and PKB/Akt control the muscle response to denervation by regulating autophagy and HDAC4. Nat Commun. 2019 Jul 18;10(1):3187

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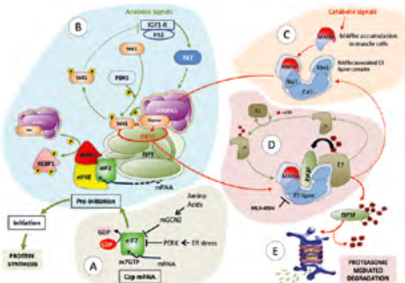

Fig. 3: Proposed model for modulation of protein synthesis in skeletal muscle. A) Assembly of capped mRNA and integration of stress signaling. **B)** EIF3F dual function: protein initiation complex (PIC) recruitment at the mRNA cap and scaffolding platform for mTORC1 and S6K1. mTORC1 affects protein synthesis via phosphorylation of 4EBP1, which thereby dissociates from eIF4E; and phosphorylation of S6K1, which facilitates the release of S6K1 from EIF3F and its activation by PDK1. **C)** Atrophy dependent induction of MAFbx leads to the formation of MAFbx E3ligase complex together with Cul1, Skp1 and Rbx1. **D)** MAFbx dependent EIF3F ubiquitination followed by **E)** proteasome mediated degradation.