Fibroblasts derived from nemaline myopathy patients: a useful cellular model for studying the pathophysiological mechanisms implicated in disease's development and for pharmacological screenings

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INTRODUCTION AND PURPOSE

Nemaline myopathy (NM) is one of the most common forms of congenital myopathy and it is identified by the presence of inclusions in muscle fibers called "nemaline bodies" or rods which are considered to be derived from Z lines because they have a similar structure and express similar proteins. Clinical features include hypotonia and muscle weakness, nevertheless, the clinical spectrum varies between lethal neonatal forms and less severe forms of later onset. This pathology is heterogeneous from a genetic point of view, its inheritance can be autosomal-dominant (AD), autosomal-recessive (AR) or sporadic. Although most cases of NM result from mutations in the genes encoding skeletal α-actin (ACTA1) and nebulin (NEB), mutations have been found in more than 10 genes that cause the disease, including genes encoding proteins for sarcomeric thin filament components (NEB, ACTA1, TPM2, TPM3, TNNT1, CFL2, LMOD3), Kelch domain-associated proteins (KBTBD13, KLHL40 and KLHL41) and an unconventional myosin (MYO18B). Unfortunately, there is no curative treatment for NM patients and the pathogenetic mechanisms remains unclear, therefore, studying fibroblasts derived from patients can be useful to understand the pathophysiological alterations of NM and to identify potential therapies.

MATERIALS AND METHODS

We study the pathophysiological alterations in NM using fibroblasts from patients with mutations in ACTA1 (Nema3 patient) and NEB genes (Nema4 patient) which were obtained by performing a skin biopsy. As a screening strategy, fibroblasts are treated with different compounds for a week and after that, they are stained with DAPI and Rhodamine-phalloidin in order to analyze the status of the cytoskeletal actin filaments by fluorescence microscopy techniques using DeltaVision system because both proteins, actin and nebulin are important components of the cytoskeleton. Positive compounds are those that improve the formation of actin filaments. Furthermore, we measure the length of actin filaments by ImageJ software in order to determine if the positive compounds are able to increase the length of the filaments. Statistically significant differences between controls and patients were determined in all experiments by Student's t test; a value of $p < 0.01$ was considered to be statistically significant.

CONCLUSIONS & FUTURE PERSPECTIVES

- Fibroblasts derived from NM patients can be a useful cellular model to study the pathophysiological mechanisms involved in NM and to find new therapies.
- Fibroblasts from NM patients with mutations in ACTA1 and NEB genes show alterations in cytoskeletal actin filaments such as smaller number and length of filaments.
- We have identified two compounds that improve the state of actin filaments in fibroblasts derived from NM patients.
- Further studies are needed to elucidate the mechanism of action of the two positive compounds identified and to determine their therapeutic applications. To study the therapeutic application of these compounds, fibroblasts derived from NM patients could be reprogrammed to muscle cells since NM patients mainly present muscular problems such as hypotonia and muscular weakness.

RESULTS

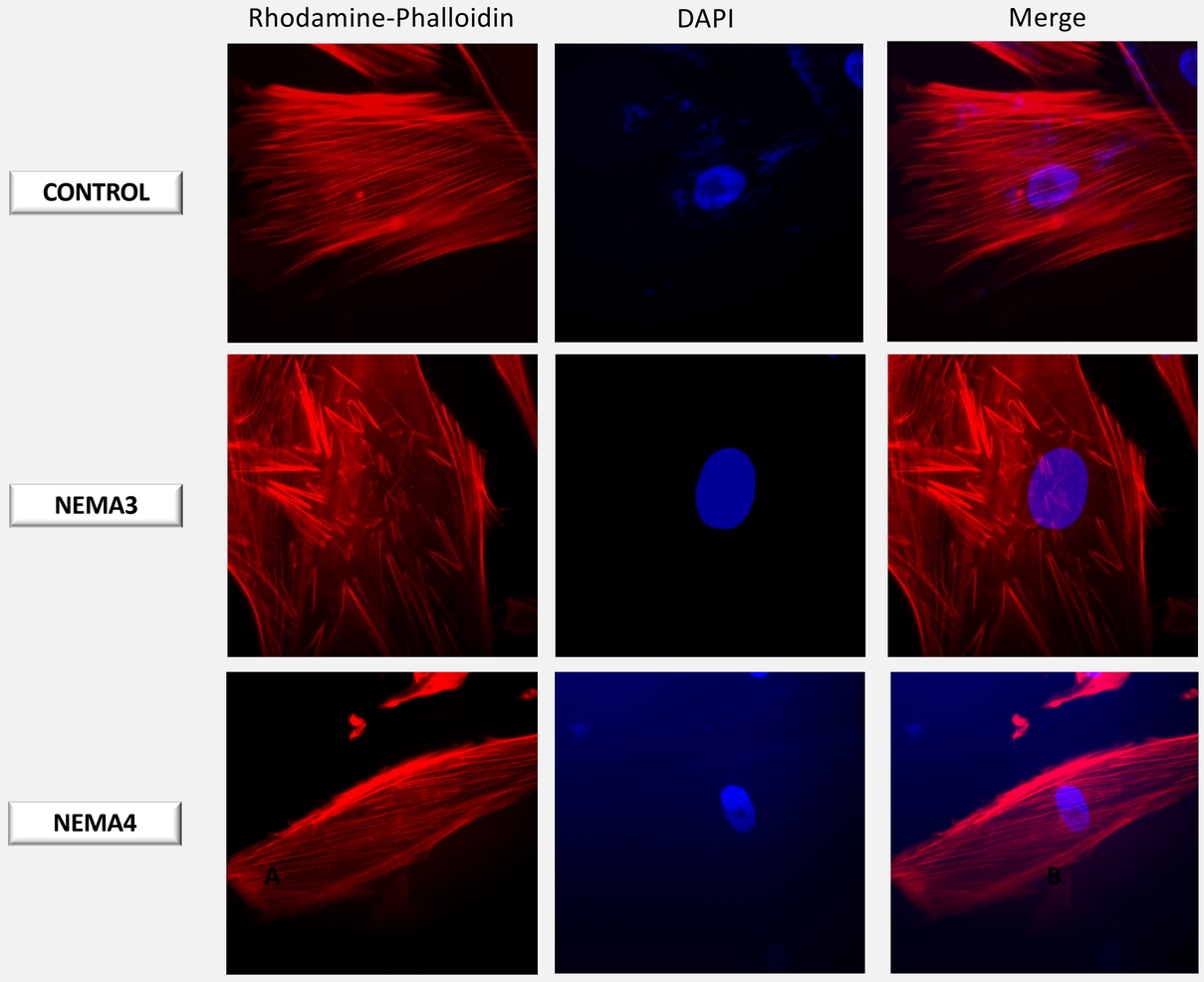

Fig. 1. DAPI and Rhodamine-Phalloidin staining of control fibroblasts and fibroblasts from NM patients. Fibroblasts from NM patients (Nema3, Nema4) showed incorrect actin filament formation compared to control fibroblasts.

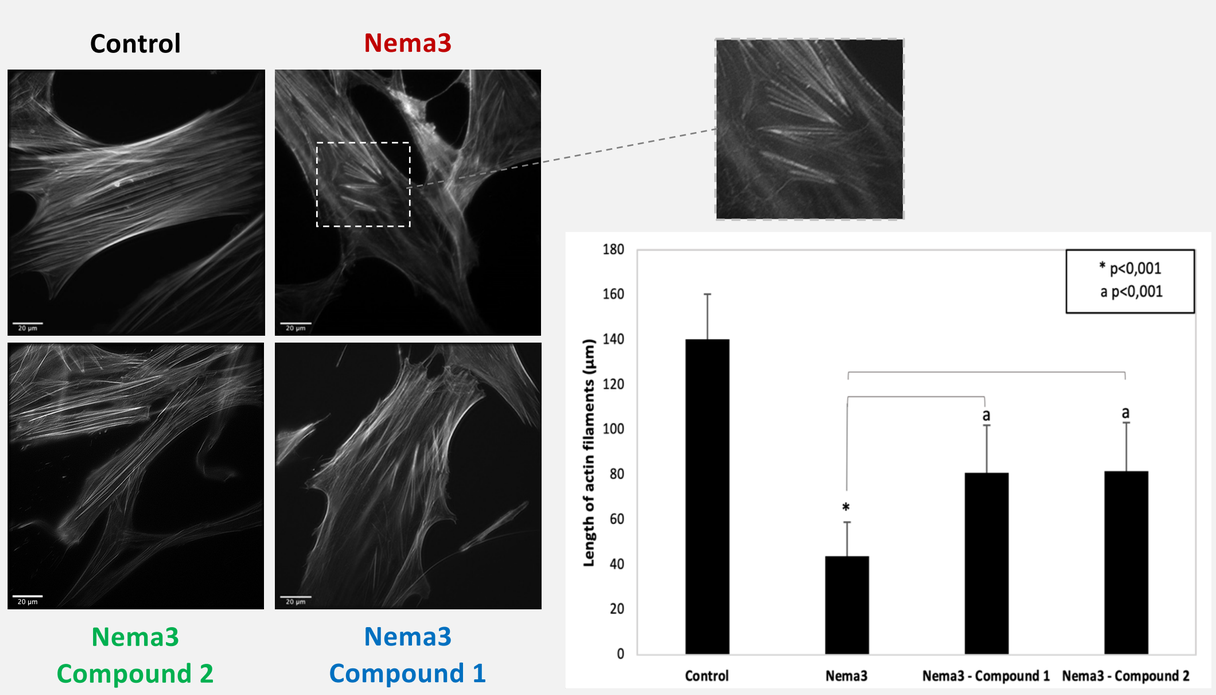

Fig. 2. A) **DAPI and Rhodamine-Phalloidin staining of control fibroblasts, fibroblasts from Nema3 patient without treatment and treated with compound 1 and 2 for a week**. B) **Measurements of actin filament length (µm)**. Treatment of fibroblasts from Nema3 patient with compounds 1 and 2 increased the length of the actin filaments.

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