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Watertown, MA 02472



TRANSLATIONAL RESEARCH

A possible approach for treating FSHD with RNAi therapeutics

Perspectives and updates from the FSH Society

Two exciting papers were recently published on possible approaches for treating FSHD using a disease gene silencing approach called RNA interference (RNAi). The details emerged within one month of each other in the journal *Molecular Therapy*. The two complementary studies were performed by different teams of scientists: the Harper Lab at The Ohio State University and Nationwide Children's Hospital in Columbus, Ohio, with a collaborator in Modena, Italy; and the Gabellini and Chamberlain labs in Milan, Italy, and Seattle, Washington, respectively.

RNA interference is a natural cellular process that controls the levels at which certain genes are expressed. In this sense, it operates less like an on/off switch and more like a molecular volume control knob. Over the last several years, many scientists have been working to co-opt these natural gene-silencing strategies for therapeutic purposes. Indeed, the main triggers of RNAi in the cell, called inhibitory RNAs or microRNAs, can be rationally designed in the lab to knock down disease genes. In the two molecular therapy studies, the research teams rationally engineered FRG1-targeted inhibitory RNAs and then delivered them to muscles of dystrophic FRG1-high mice using adeno-associated viral (AAV) vectors, which are benign in humans. FSHD Region Gene 1, otherwise known as FRG1, is a gene that is very near the deleted region of DNA associated with FSHD called the D4Z4 region and it has been widely studied as a possible candidate gene for FSHD.

The first of these studies, published by Wallace, et al., on July 5, 2011, reported that AAV-delivered artificial microRNAs reduced toxic FRG1 levels and improved histological and functional muscle abnormalities associated with FRG1 over expression in mice. Drs. Wallace and Harper write that since this disease allele-specific gene silencing using RNAi is feasible, this "work supports that RNAi-based gene therapy is a promising candidate strategy for treating dominant myopathies, regardless of the causal genetic mutation." The Harper Lab is currently modifying this strategy to target another FSHD candidate gene, DUX4, as well as genes involved in other dominant muscular dystrophies, including some forms of Limb Girdle Muscular Dystrophy (LGMD).

In the second study, published on August 9, 2011, Bortolanza, et al., note that administering shRNA, "with a single, systemic delivery they reached all the skeletal muscles body-wide and obtained a specific and long term FRG1 silencing. This was associated to a significant rescue of the phenotype at histological, molecular and functional levels. Importantly, there was no sign of toxicity. More importantly, they treated adult animals that had already developed signs of muscular dystrophy to closely mimic possible future clinical settings. While in the paper we targeted the FRG1 gene,

the same approach is easily applicable to DUX4 knockdown by simply exchanging the shRNA expression cassette. Hence our approach is applicable to any FSHD candidate gene.”

Both papers, authored by FSH Society funded researchers, are important for several reasons. First, they are the first successful proof of concept of a possible therapeutic approach for FSHD. Second, the approach is relevant for dominant myopathies in general. Mutations in at least 29 genes are responsible for a variety of genetically dominant muscle diseases. Considered as a group, these may affect as many as 1 individual in 2,400, making them the most common muscle disorders. Indeed, of the three most important muscle diseases (FSHD, Myotonic and Duchenne), FSHD and myotonic are genetically dominant disorders. Nevertheless, dominant myopathies have been largely neglected as targets of translational research because feasible molecular approaches for suppressing disease genes were unavailable until RNAi emerged a few years ago. Based on their results, these researchers predict that approaches similar to the one that they described could be applicable (with modifications depending on the specific disease) to a large number of patients affected by dominant myopathies. The financial and other support of the FSH Society was very important in obtaining the results described in these papers.

Drs. Gabellini, Harper, Wallace, Garwick-Coppens and Tupler are all past or current FSH Society fellows. Drs. Gabellini, Harper and Wallace began their careers in FSHD with grants from the FSH Society. While we remain cautiously optimistic about a treatment for FSHD, as the verification, corroboration and identification of the FSHD target (gene, RNA, protein) continues, this work is a major step forward in proof of concept of RNAi therapy in FSHD models. This work was made possible by a culmination over the years of a combination of FSH Society Marjorie and Gerald Bronfman fellowship grants, Jacobs Family and Friends research fellowship grants, FSH Society Grant Delta Railroad Construction Company fellowship grant and a FSH Society Landsman Charitable Trust fellowship grant.

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RNA Interference Improves Myopathic Phenotypes in Mice Over-expressing FSHD Region Gene 1 (FRG1)

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Wallace LM, Garwick-Coppens SE, Tupler R, Harper SQ

Molecular, Cellular, and Developmental Biology Graduate Program, The Ohio State University, Columbus, Ohio, USA. Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA.

Muscular dystrophies, and other diseases of muscle, arise from recessive and dominant gene mutations. Gene replacement strategies may be beneficial for the former, while gene silencing approaches may provide treatment for the latter. In the last two decades, muscle-directed gene therapies were primarily focused on treating recessive disorders. This disparity at least partly arose because feasible mechanisms to silence dominant disease genes lagged behind gene replacement strategies. With the discovery of RNA

interference (RNAi) and its subsequent development as a promising new gene silencing tool, the landscape has changed. In this study, our objective was to demonstrate proof-of-principle for RNAi therapy of a dominant myopathy in vivo. We tested the potential of adeno-associated viral (AAV)-delivered therapeutic microRNAs, targeting the human Facioscapulohumeral muscular dystrophy (FSHD) region gene 1 (FRG1), to correct myopathic features in mice expressing toxic levels of human FRG1 (FRG1(-high) mice). We found that FRG1 gene silencing improved muscle mass, strength, and histopathological abnormalities associated with muscular dystrophy in FRG1(-high) mice, thereby demonstrating therapeutic promise for treatment of dominantly inherited myopathies using RNAi. This approach potentially applies to as many as 29 different gene mutations responsible for myopathies inherited as dominant disorders.

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<http://www.nature.com/mt/journal/vaop/ncurrent/full/mt2011118a.html>

The following are selected excerpts from Wallace, Harper et al paper's Discussion section: "We used the FRG1-high mouse model in this study, which was initially developed to test the hypothesis that FRG1 over expression was a primary pathogenic insult underlying FSHD. Although the progressive myopathy produced in these mice strongly supported this hypothesis, there have been some conflicting data arguing against the involvement of FRG1 in FSHD, or at least minimizing its role as a primary pathogenic insult. Thus, it is fair to say that FRG1 is a controversial FSHD candidate gene. Nevertheless, for this study, we were unconcerned with this ongoing debate, because our primary goal was to demonstrate proof-of-principle for RNAi therapy of dominant myopathies in general, and the FRG1-high line was useful as an outstanding model of dominant muscle disease. We reasoned that its involvement in FSHD, or lack thereof, was irrelevant to the goal of this study. We therefore developed a gene therapy strategy to knockdown pathological levels of human FRG1 in FRG1-high mouse muscles. Here, we reported that AAV6-delivered artificial microRNAs reduced toxic FRG1 levels and improved histological and functional muscle abnormalities associated with FRG1 over expression in mice. Our work therefore supports the therapeutic potential of RNAi therapy for dominant myopathies in general. In addition, it could be applied to FSHD, if additional evidence supporting FRG1 involvement in the disease emerges; alternatively, our strategy could be modified to target other FSHD candidate genes, such as DUX4."

AAV6-mediated Systemic shRNA Delivery Reverses Disease in a Mouse Model of Facioscapulohumeral Muscular Dystrophy

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Bortolanza S, Nonis A, Sanvito F, Maciotta S, Sitia G, Wei J, Torrente Y, Di Serio C, Chamberlain JR, Gabellini D

Dulbecco Telethon Institute and Division of Regenerative Medicine, San Raffaele Scientific Institute, Milano, Italy

Abstract

Treatment of dominantly inherited muscle disorders remains a difficult task considering the need to eliminate the pathogenic gene product in a body-wide fashion. We show here that it is possible to reverse dominant muscle disease in a mouse model of facioscapulohumeral muscular dystrophy (FSHD). FSHD is a common form of muscular dystrophy associated with a complex cascade of epigenetic events following reduction in copy number of D4Z4 macrosatellite repeats located on chromosome 4q35. Several 4q35 genes have been examined for their role in disease, including FRG1. Overexpression of FRG1 causes features related to FSHD in transgenic mice and the FRG1 mouse is currently the only available mouse model of FSHD. Here we show that systemic delivery of RNA interference expression cassettes in the FRG1 mouse, after the onset of disease, led to a dose-dependent long-term FRG1 knockdown without signs of toxicity. Histological features including centrally nucleated fibers, fiber size reduction, fibrosis, adipocyte accumulation, and inflammation were all significantly improved. FRG1 mRNA knockdown resulted in a dramatic restoration of muscle function. Through RNA interference (RNAi) expression cassette redesign, our method is amenable to targeting any pathogenic gene offering a viable option for long-term, body-wide treatment of dominant muscle disease in humans.

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<http://www.nature.com/mt/journal/vaop/ncurrent/full/mt2011153a.html>

The following are selected excerpts from Bortolanza, Gabellini et al paper's Discussion section: "Although several intriguing FSHD candidates have been proposed, no single gene has been conclusively linked to FSHD development thus far. It was reported that the D4Z4 repeat contains an ORF encoding a double homeobox protein named DUX4. DUX4 has been detected in FSHD-derived primary myoblasts but not in controls, suggesting that D4Z4 may directly affect disease progression through the aberrant production of DUX4. Several functional studies described extreme general toxicity for DUX4. Cellular toxicity of DUX4 coupled with very low DUX4 expression in human cells poses a difficult challenge for modeling the human disease in mice. However, if a DUX4 mouse model is produced, our approach could be adapted for DUX4 knockdown in muscle through retargeting of the RNAi hairpin sequence to DUX4 mRNA.

"A growing understanding of its function, strongly suggests that FRG1 overexpression plays an important role in FSHD. Based on these data, FRG1 inhibition would be expected to lead to a therapeutic benefit in FSHD. Hence, we have used the only available FSHD mouse model to provide a proof of principle with respect to the use of RNAi therapeutic approaches for FSHD. In this study, we demonstrated long-term, dose-dependent reduction in FRG1 expression in all the muscles analyzed. Therapeutic benefits were observed in all of the mice treated with either low or high AAV6-sh1FRG1 doses

"In conclusion, we have shown that we can prevent disease progression with systemic, AAV6-mediated FRG1 RNAi performed following disease onset. This work exemplifies the power and specificity of RNAi in a widespread tissue in a living animal and offers a potential route to clinical application and treatment for individuals who are already showing symptoms of disease. The efficient, stable, long-term therapeutic reduction of pathological signs in the FRG1 mouse suggests the added potential clinical benefit of

efficacy with limited dosing. This therapy allowed significant improvement of disease and could potentially be translated to human patients. The knowledge gained through these studies could facilitate the development of new therapies to treat FSHD and other dominant diseases.”

For more information please contact:

Daniel Paul Perez
President & CEO
FSH Society, Inc.
64 Grove Street
Watertown, MA 02472 USA
(781) 275-7781, (617) 658-7811
(781) 275-7789 or (617) 659-7811 fax
daniel.perez@fshsociety.org