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Continuing to make progress in understanding and treating FSHD
Grant awards for August 2016 cycle

Since 1998, the FSH Society has transformed FSHD research by providing grants for vital start-up funding for investigators in FSHD and research projects on FSHD. The FSH Society has two rounds of grant applications each year, with deadlines in February and August. Grant applications are thoroughly analyzed and vetted by the SAB. An initial letter of intent is submitted, which is reviewed by Professor David Housman, Chair of the SAB. If a letter of intent is accepted, the applicant submits a full application. The main section where researchers describe the proposed work and workflow is around 12 pages long.

Upon receipt of all full grant applications for a particular round, Professor Housman assigns teams of two or more members of the SAB to critique each proposal. Any potential conflicts of interests are noted, and SAB members who may have a conflict are not assigned to review, and do not vote on, the particular proposal. The two reviewers review the application in depth and provide a detailed written description and recommendation to the other members. Initial critiques are due within three weeks of the assignment and a full meeting of the SAB is held around two weeks thereafter. Grant applications are reviewed and voted upon by the entire SAB, with discussion led by the two primary reviewers. SAB recommendations for approved applications are then sent to the Society's Board of Directors for a vote. When the SAB disapproves an application, it provides the applicant with a detailed description of the reasons for disapproval, and the applicant may resubmit the application for consideration in a later round. SAB members and the chair serve without pay.

Upon acceptance by the Society's board, the grantee receives a letter of acceptance and a grants policies and procedures document. The grantee is then asked for written confirmation indicating their intention of accepting or declining the fellowship knowing that the grant is administered in accordance with the FSH Society's policies document. It is understood that the funds awarded have not been provided for any other purpose than research on FSHD. The grantee is asked to reply within two weeks where upon a check is issued in advance for the first six months with equal installments to follow at subsequent six month intervals based on review of requested progress reports.

The milestones and insights gained are significant. The fellowship program allows innovative and entrepreneurial research to develop, prove successful, and ultimately to attract funding from large funding sources such as the US National Institutes of Health (NIH) and large private sources.

On January 23, 2017, the Scientific Advisory Board (SAB) of the FSH Society, chaired by David Housman, Ph.D., held its grant review. The SAB reviewed the grant applications and progress reports for the August 2016 round. The SAB made recommendations, gave guidance and indicated if additional information was needed or if action needed to be taken. The SAB gave a ranking by majority consensus. The meeting was recorded and was transcribed. By February 7, 2017, the FSH Society Board of Directors reviewed and approved the SAB recommendations for funding. Below is a list of the funded projects, including project descriptions as submitted by the applicants. For the August 31, 2016, round of grant applications eight grant applications were received; five were funded in the amount of $541,133.71.

We are very pleased to list the grantees funded in the August 2016 cycle.

August 2016 cycle

1. FSH Society-NDRI Tissue Procurement Project

Tom Bell, M.D., Ph.D.
Jeffrey Thomas, Ph.D.
Denee Tidwell
National Disease Research Interchange, Philadelphia, Pennsylvania, USA
03/15/2017 - 03/14/2018
$38,202 for one year + $26,500 for tissue recovery costs
FSHS-82016-01 cont. FSH Society Grant FSHS-22015-08

Project Summary

In response to a request from the FSH Society, NDRI proposes to develop and implement a resource to recover surgical and post mortem human bio specimens and distribute them to approved investigators. This resource will utilize NDRI's experience, expertise and established systems to expand and enhance the type, number and quality of human tissues available to the FSH research community. lt is proposed that NDRI's Private Donor Program will collaborate with FSH to recover and distribute tissues from patients who participate in the FSH Registry and who have provided consent for the recovery of tissues and organs for research.

In addition to providing all resources required to recover tissues post mortem and from surgical procedures, NDRI will provide informational materials to the FSH Society for distribution to potential registry participants, as well as IRS-approved templates for obtaining informed consent from patients and authorization to donate from family decision makers.

2. Inhibited protein turnover and TDP-43 aggregation in FSHD pathogenesis

Sachiko Homma, Ph.D., Boston University, Boston, Massachusetts USA
Jeffrey Miller, Ph.D., Boston University, Boston, Massachusetts USA
03/15/2017 - 03/14/2018
$58,920 for one year
FSHS-82016-02 cont. FSH Society Grant FSHS-22015-01

Project Summary

Pathogenesis in Facioscapulohumeral muscular dystrophy (FSHD) appears to be due to aberrant expression, particularly in skeletal muscle nuclei, of the full-length isoform of DUX4 (DUX4-FL). DUX4-FL had been shown to induce toxicity by ectopic expression and can lead to aberrant expression of DUX4-FL target genes including ubiquitin ligases, ubiquitin binding proteins, and RNA processing genes as well as germline and stem cell genes (1-6). We and another group identified disturbed proteostasis as a possible mechanism for DUX4-mediated pathology (7, 8). Dysregulation of proteostasis can interfere with normal cellular functions, cause stress or immune responses, and lead to disease. Abnormal RNA or protein accumulation has been implicated in a number of diseases including amyotrophic lateral sclerosis (ALS), inclusion body myositis (IBM), and other myopathies (9-14). DUX4 expression also inhibits nonsense-mediated decay (NMD) (8), which can lead to abnormal expression, processing, or accumulation of RNAs and protein, including DUX4 itself. We discovered that DUX4-FL, but not DUX4-S, inhibits protein turnover and leads to abnormal ubiquitin expression and nuclear aggregation of TDP-43 (TAR DNA-binding protein 43), one of the aggregation-prone and RNA/DNA binding proteins previously associated with ALS and IBM (7). Importantly, the abnormal deposition of ubiquitinated protein and nuclear aggregation of TDP-43 were observed when DUX4-FL was expressed from its endogenous promoter, as well as when it was exogenously expressed. For this project, we hypothesized that DUX4-FL expression would induce progressive impairment of the ubiquitin-proteasome system (UPS). We proposed, therefore, to identify mechanisms that underlie the DUX4-FL-induced dysregulation of proteostasis and protein

aggregation as a step to understanding pathogenesis and developing therapeutic strategies for FSHD. The two specific aims are to 1: Identify the mechanisms by which DUX4-FL inhibits protein turnover and 2: Determine if FSHD muscle tissues show signs of disturbed proteostasis. As in one-year progress report and request for funding extension, we have made significant progress towards accomplishing both aims.

3. Dynamic Mapping of Perturbed Signaling Underlying FSHD

Peter Zammit, Ph.D., King's College London, London England, UK
Chris Banerji, Ph.D., King's College London, London England, UK
03/15/2017 - 03/14/2018
$83,207.71 for one year
FSHS-82016-03 cont. FSH Society Grant FSHS-82013-06 [3rd year]

Project Summary

Facioscapulohumeral muscular dystrophy (FSHD) is an adult-onset, autosomal dominant disorder initially characterised by wasting of facial muscles and upper body musculature. Disease can progress to affect muscles of the lower extremities and severely impair quality of life. Over 95% of FSHD cases are classed as FSHD1, caused by contraction to less than 11 units of the D4Z4 microsatellite repeat, on the subtelomeric region of chromosome 4. At least one D4Z4 unit is required to cause FSHD however, and only when inherited with a specific polymorphism on the distal end of chromosome 4 (e.g. 4qA161). Each D4Z4 unit contains an open reading frame for the double homeobox 4 (DUX4) retrogene, with specific 4qA haplotypes providing a polyadenylation signal for DUX4 transcripts generated by the last D4Z4 unit. This permissive chromosomal configuration generates stable DUX4 transcripts and FSHD is caused by a toxic gain-of-function of DUX4. FSHD2 is caused by mutation in genes responsible for methylation at D4Z4, with the resulting hypomethylation again causing DUX4 expression, but without contraction at D4Z4.

FSHD myoblasts are particularly sensitive to oxidative stress. Thus treatment by anti-oxidants has been explored as a therapy. A recent clinical trial (clinicaltrials.gov number: NCT01596803) administered vitamin E, vitamin C, zinc, and selenomethionine to FSHD patients for 17 weeks to enhance anti-oxidant defense and reduce oxidative stress. They reported improved maximal voluntary contraction and endurance limit time in quadriceps muscle of the treated patients, but no effects on the two-minute walking test (Passerieux et al. 2014 - doi:10.1016/j.freeradbiomed.2014.09.014).

To identify pathways that lead to compromised muscle function, we have performed RNA-Seq on cell lines derived from FSHD patients in a high-frequency time course of genome wide gene expression during myogenic differentiation (funded by the FSHSociety). Using mathematical methodologies with optimised network theoretic tools on this gene expression dataset, will have revealed molecular mechanisms of myogenesis in FSHD. This analysis of our RNA-Seq time course data has led to a number of novel insights into FSHD molecular mechanisms, particularly implicating critical mediators of oxidative stress, mitochondrial biogenesis, the TCA cycle and myogenic progression, as perturbed in FSHD.

Our RNA-Seq data indicated suppression of mitochondrial biogenesis during FSHD myogenesis, and mitochondrial dysfunction has been reported in FSHD, indicating that activation of this pathway could provide a therapeutic strategy. For rapid translation to the patient/clinic, we have investigated nutritional supplements that target this pathway and found that several improve myogenesis of FSHD patient derived cells. In this project, we will screen several more nutritional supplements and select the most promising for testing in a wide range of different FSHD patient derived myoblasts. We will also test selected nutritional supplements on myoblasts expressing DUX4, to determine their effectiveness at ameliorating the drastic phenotype elicited by DUX4. Effects of nutritional supplements on signaling pathways that are perturbed in FSHD will also be examined to better understand their mechanism of action.

By analyzing modifiers of these pathways we hope to improve our understanding of the molecular defects in FSHD and how best to modify them to maximize patient benefit. Ultimately the aim is translation of

such an approach to a clinical trial setting, and as we focus on nutritional supplements, it is likely that such translation could be rapid.

4. Developing LNA-based therapy for facioscapulohumeral muscular dystrophy

Yi-Wen Chen, D.V.M., Ph.D.
Children's National Health System, Washington DC, USA
Toshifumi Yokota, Ph.D.
University of Alberta Faculty of Medicine and Dentistry Alberta, Canada
04/01/2017 - 03/31/2019
$179,104 for two years
FSHS-82016-04

Project Summary

Facioscapulohumeral muscular dystrophy (FSHD) is believed to be caused by the aberrant expression of double homeobox protein 4 (DUX4) due to epigenetic changes at chromosome 4q35 region. Antisense oligonucleotide (AON) therapy is a promising strategy to eliminate pathogenic gene product, such as DUX4 mRNA, in cells. In this study, we will investigate one of promising AON compounds called LNA gapmer for its efficacy in reducing DUX4 in cell culture and in a mouse model of FSHD. The findings will allow us to evaluate this compound as a potential treatment for FSHD. Antisense therapy shows promise for treating an array of disorders, however, several problems associated with AONs yet to be improved, including 1) difficult in systemic drug delivery because these AONs could not easily cross the lipid bilayer of cells; 2) harmful off-target effects and immune responses via toll-like receptors; 3) low stability due to degradation by intracellular and extracellular nucleases. Considering these challenges, locked nucleic acids (LNAs) show exceptional thermal stability, impose significant protection against nucleolytic degradation and have a high binding affinity. Importantly, LNA can be systemically delivered in vivo. Modifications to the LNA gapmer chemistry have also shown great success and allow RNase H-mediated cleavage to degrade target RNAs. In our preliminary study, we designed LNA gapmers targeting DUX4 and successfully knocked down DUX4 mRNA in immortalized FSHD myoblasts. The goal of this study is to further characterize LNA gapmers for its efficacy and safety in vitro and in vivo. The studies will be conducted by two highly experienced investigators with complementary expertise in the field. Dr. Toshifumi Yokota who is an expert in AON therapy and has designed and generated the in vitro preliminary data in collaboration with Dr. Chen. Dr. Yi-Wen Chen has extensive experience in FSHD and FSHD mouse models. Dr. Yokota will be in charge of designing the LNA gapmers and performing in vitro studies using immortalized FSHD myoblasts as proposed in Aim 1. Dr. Chen will be in charge of performing in vivo studies using a new mouse model of FSHD to determine the efficacy of the LNA gapmers in vivo as proposed in Aim 2. The two investigators have been closely working together to develop this study and will keep the collaborative nature of work during the funding period. The goal is to carefully characterize the LNA gapmers that target DUX4 and identify those with the highes efficacy and specificity for treatment development. There is no effective treatment for FSHD, however, aberrant expression of DUX4 is known to cause this disorder. The proposed studies will study an effective antisense oligonucleotide strategy to target DUX4 and reduce its expression. LNA has been studied in vitro and in vivo for efficacy and safety extensively. We have generated preliminary data to show effective DUX4 knockdown in FSHD myoblasts. This collaborative study will characterize the LNA Gapmers against DUX4 further as potential therapeutics for FSHD.

5. Activity of estrogen on FSHD muscle differentiation

Fabiola Moretti, Ph.D.
Institute of Cell Biology and Neurobiology - National Research Council of Italy (CNR), Rome, Italy
03/15/2017 – 03/14/2019
$155,200 for two years

FSHS-82016-05

Project Summary

Facioscapulohumeral muscular dystrophy (FSHD) is characterized by extreme variability in symptoms with females being less severely affected than males and presenting a higher proportion of asymptomatic carriers. Thus far, gender factors involved in the disease have not been identified. Recent data from our group demonstrate that estrogens improve in vitro the differentiation ability of myoblasts from FSHD patients without affecting cell proliferation or survival. Specifically, estrogens counteract the muscle differentiation impairment caused by the homeobox protein DUX4, the best FSHD candidate gene. We further demonstrated that estrogen receptor beta (ERβ), present in female and male individuals, is involved in this activity by displacing DUX4 from the nucleus and impairing its transcriptional function. Importantly, both 17β-estradiol, the predominant female hormone regarding estrogenic activity, as well as 5α-Androstane-3β,17β-diol (3β-diol), a natural endogenous ligand of ERβ present in males, can promote this ERβ-mediated activity.

The present project aims to confirm these data in vivo by analyzing the effect of estrogen on the ability of transplanted human muscle-derived cells to participate in the regeneration of injured muscle in immune-deficient mice. The choice of this model is based on the following reasons:

1.) The role of muscle differentiation defects in the pathophysiology of FSHD is still controversial. Conversely, muscle degeneration with fatty replacement in humans has been shown as well as impaired regeneration in FSHD mouse models;

2.) the ability of FHSD muscle-precursor cells as mesoangioblasts, or of FSHD myoblasts to differentiate into skeletal muscle in immunodeficient mice has been previously reported;

3.) it has been recently reported an innovative approach for the generation of a mature skeletal muscle based on a hydrogel/growth factor scaffold. Based on these data, we reasoned that the model of muscle regeneration is a suitable model to test the beneficial effect of estrogen in vivo.

Given the low proliferative potential of myoblasts, we will use muscle-precursor cells (perivascular cells). Specifically, we propose to transplant human perivascular cells expressing exogenous or endogenous DUX4 in muscle-ablated mice and analyze their ability to form myotubes depending on the levels and/or activity of estrogens. The project includes three main tasks:

1.) analyze the effect of estrogen on transplanted human muscle-precursor cells. This task will establish the experimental conditions for further analyses: - test the growth of PVCs dependent on the gender and the levels of different estrogenic compounds; - test the best conditions for growth of exogenous DUX4-expressing PVCs.

2.) analyze the regeneration ability of DUX4Cherry-PVCs depending on the levels/activity of estrogens. This task is the core of the project, aiming to analyze the regeneration ability of DUX4-expressing cells depending on the levels/activity of estrogen. Different groups of animals will be used based on gender difference and/or estrogen levels or activity (using specific estrogen antagonist able to bind ERβ and to compete with natural endogenous ligands).

3.) analyze the regeneration ability of FSHD-derived PVCs. PVCs derived from FSHD patients will be subjected to the same protocol of transplantation and their myofiberformation ability tested. Given the preciousness of FSHD muscle biopsy, based on the results of the previous task, FSHD-derived PVCs will be challenged in the most efficient conditions.

This project will ascertain the in vivo role of estrogens towards FSHD, particularly on the regeneration ability of DUX4-expressing PVCs. The success of this project will support the estrogen as one of the factors underlying FSHD gender differences and will establish their protective function against this disease. Most importantly, these data might open the venue to therapeutic intervention in FSHD patients.