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Continuing to make progress in understanding and treating FSHD
Grant awards for February 2017 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 May 30 and June 1, 2017, the Scientific Advisory Board (SAB) of the FSH Society, chaired by David Housman, Ph.D., held its biannual review of grant applications. The SAB reviewed new grant applications, resubmitted grant applications, applications requesting continuations for the February 2017 round and progress reports. By July 5, 2017, the FSH Society Board of Directors reviewed and approved the FSH Society's SAB, the Society's Science, Technology and Research (STaR), and, Finance Committees' recommendations for funding. Below is a list of the funded projects, including project description as submitted by the applicant. For the February 28, 2017 round of grant applications, we received ten applications (six new, one resubmission) and three requests for one year extension on ongoing research projects. Eight were awarded; two were rejected. One was declined by grant awardee due to receiving another grant resulting in overlapping funding sources. Seven were funded in the amount of US$616,476.

We are very pleased to list the projects and grantees funded in the February 2017 cycle.

February 2017 Cycle

1. A genome-wide CRISPR knock-out strategy to identify modifiers of FSHD
Angela Lek, Ph.D., Genetics and Genomics, Boston Children's Hospital, Boston Massachusetts USA
Louis Kunkel, Ph.D., Genetics and Genomics, Boston Children's Hospital, Boston Massachusetts USA
08/01/2017- 07/31/2018
US$75,860 for one year
FSHS-22017-01 [cont. FSH Society Grant FSHS-82015-04]

Project Summary

Facioscapulohumeral dystrophy (FSHD) is a common but unique form of muscular dystrophy requiring multiple factors to create a 'permissive' state for disease manifestation. Over recent years, several genetic (DUX4) and epigenetic (hypo-methylation) factors have been linked to FSHD pathogenesis; however, it has become clear that the field has not elucidated all factors required for disease manifestation. Mounting clinical evidence suggests the existence of modifier genes with the capacity to regulate DUX4 transcript and/or protein function. Recent advances in genome-editing technologies proposed for use in this project now should enable us to uncover these remaining missing links. Through the systematic introduction of loss-of-function mutations into genomic DNA, we can interrogate the genome for answers that may explain the phenotypic variability between patients, as well as the non-penetrant effects of DUX4 in some individuals. In this project, we propose a targeted genome-scale knock-out screen to identify genes that can reduce the phenotypic impact of DUX4 expression when inactivated. We hypothesize that there exists gene targets of DUX4 whose loss will render DUX4 unable to trigger a dysregulated cascade of gene expression, thus abrogating its toxicity. These candidates likely serve as genetic modifiers of FSHD, and will be readily identified by downstream sequencing and computational analysis for detection of CRISPR target genes enriched within these DUX4 'resistant' cell populations. This will allow the generation of a complete list of gene candidates with the potential to influence the pathogenic outcomes associated with DUX4 misexpression. Identified gene hits will be cross-referenced to our whole-genome sequencing data of nonmanifesting carriers to search for sequence variants that may enable us to narrow down promising candidates for functional follow up studies. Validation of candidate modifier genes will be performed in our established zebrafish model of FSHD for rescue of phenotype to confirm functional significance. Additionally, we will revert to our repository of FSHD patient cells to genome edit our candidate genes under these permissive allelic conditions, and subsequently measure changes in known FSHD biomarker expression. FSHD is a challenging disease whose remaining unanswered questions cannot be accomplished alone. Hence, our proposal involves a multi-institute collaboration, bringing together a wealth of patient resources (Wellstone Center), the latest in genomic technology (Broad Institute), and a well-established animal model of FSHD (Boston Children's Hospital). Not only will the identification of these modifier genes for DUX4 resistance provide valuable insights into FSHD disease pathogenesis, but they will also present as solid leads that can be directly targeted for therapeutic intervention in humans with FSHD.

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2. Determining the effectiveness of increased SMCHD1 expression to suppress DUX4 in FSHD muscle cells and model mice
Yosuke Hiramuki, Ph.D., Fred Hutchinson Cancer Research Center, Seattle, Washington USA
Stephen Tapscott, M.D., PhD., Fred Hutchinson Cancer Research Center, Seattle, WA USA (mentor)
9/23/2017-9/22/2018 for one year
US$53,520
FSHS-22017-02 [cont. FSH Society Grant FSHS-22015-02]

Project Summary

Facioscapulohumeral muscular dystrophy (FSHD) has two types, FSHD1 and FSHD2. The causative gene of FSHD1 and FSHD2 is DUX4. The reasons that DUX4 is expressed in FSHD1 and FSHD2 are

contraction of the D4Z4 macrosatellite repeat unit and mutations in SMCHD1, respectively, combined with 4qA allele carrying the DUX4 polyadenylation site. In addition, SMCHD1 modifies disease severity in families affected by FSHD1. Here, to understand the molecular mechanisms to express DUX4, I seek to identify how SMCHD1 is involved in DUX4 regulation (Aim1 and Aim2). Moreover, I seek to elucidate the role of Smchd1 in FSHD1 model mice (Aim3).

Aim1. Identification of the molecular mechanism used by the mini-SMCHD1 to derepress DUX4 expression.

Mini-SMCHD1 (Exon1-9.41-48) de-represses DUX4 expression. To clarify the mechanisms of the increased DUX4 expression by mini-SMCHD1 in FSHD1 myoblasts, I test whether the mini SMCHD1 decreases the amount of endogenous SMCHD1 protein and/or whether the mini SMCHD1 binds to the D4Z4 region and displace the endogenous SMCHD1.

Aim2. Identification of the molecular mechanism of SMCHD1 cleavage.

There could be full-length and cleavage fragment bands on endogenous SMCHD1 in control and FSHD1 muscle cells. My hypothesis is that SMCHD1 that lacks putative cleavage sites could be more stable than endogenous SMCHD1 and it has better ability to repress DUX4 expression. To test this, I will first identify which sequence is recognized by which protease with bioinformatics and molecular biology tools. Next, I will investigate whether the protease and its recognition sites involved in the cleavage of SMCHD1 could be a novel target for therapeutic intervention.

Aim3. Identification of the role of Smchd1 in FSHD1 model mice.

Decreased SMCHD1 level de-represses DUX4 expression in FSHD1 myoblasts. To investigate whether Smchd1 could affect DUX4 expression in FSHD1 model mice, I will compare DUX4 expression in Smchd1 conditional knockout mice *(D4Z4-2.5; Myf5Cre/+; Smchd1flox/flox)* with that in control mice *(D4Z4-2.5; Myf5+/+; Smchd1flox/flox)* both under injury condition as well as normal mature muscle.

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3. SMCHD1 an epigenetic key player of chromatin regulation in two unrelated disease: FSHD and BAMS syndrome

Camille DION, Ph.D. Student, INSERM UMR_S910, Aix Marseille Universite, FRANCE
Frederique Magdinier, Ph.D., INSERM UMR_S910, Faculté de médecine de la Timone, Aix Marseille Universite, FRANCE (mentor)
US$25,000
10/01/2017-03/30/2018 for six months
FSHS-22017-04

Project Summary

For FSHD the current model suggests that a shortened D4Z4, subsequent DNA hypomethylation at a permissive 4qA allele induce expression of the DUX4 transcript that in turn activate other genes leading to the muscle-specific phenotype. For a small subset of patients (approximately 5%, FSHD2), the clinical phenotype is identical but appears without D4Z4 array contraction. However, most of these patients display a profound D4Z4 hypomethylation linked to some of them to mutations in the SMCHD1 (Structural Maintenance of Chromosomal Hinge Domain Containing) gene. Epigenetic alterations are thus closely associated to FSHD but the underlying mechanisms remain unclear and the functions of the SMCHD1 protein in the regulation of D4Z4 remains partly understood.

SMCHD1 is a large 230 kDa non-canonical member of the SMC family of chromosomal proteins. The main conserved domains are the carboxy-terminal SMC hinge domain flanked by short coil-coiled regions, the amino-terminal GHKL ATPase domain and a region with weak homology to the Bromo-adjacent homology (BAH) domain near the ATPase domain. SMCHD1 is able to homodimerize through the SMC hinge domain and is preferentially loaded on H3K9m3- enriched chromatin. In the mouse, Smchd1 is mainly characterized for its implication in X chromosome inactivation. Smchd1 is also involved in silencing of repetitive DNA sequences, regulation of clustered imprinted genes and of the monoallelically expressed Protocadherin genes cluster. SMCHD1 has also been found at telomeres with a direct correlation

between telomere length and SMCHD1 enrichment. However, its precise role at telomeres is unknown. The aim of this project is to understand the role of SMCHD1 in chromatin regulation and DNA methylation during muscular differentiation to uncover how this protein contributes to the physiopathomechanisms underlying the FSHD disease. To this aim, we will use induced pluripotent stem cells from patients affected with FSHD1 and FSHD2 carrying different SMCHD1 mutation. We have developed in the team a strategy for the production of skeletal muscle cells from hiPSCs (myoblastes and myotubes) which will be use to monitor the expression of genes dysregulated during differentiation and to determine the profile of SMCHD1 binding to chromatin.

The goal of this project is to identify pathways dysregulated in the disease in order to get further insights into the disease pathomechanism.

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4. Skeletal muscle degeneration in the iDUX4pA mouse model
Michael Kyba, Ph.D., University of Minnesota, Minneapolis, Minnesota USA
US$100,000 total ($50,000 annually)
8/1/2017 - 7/31/2019 for two years
FSHS-22017-05

Project Summary

Facioscapulohumeral muscular dystrophy (FSHD) a genetically dominant progressive muscular dystrophy associated with derepression of the DUX4 gene. One of the major current roadblocks to FSHD basic research and therapeutic testing is the lack of a suitable animal model, with existing attempts either being too severe or lacking a muscle disease entirely. We have developed a new transgenic mouse with tissue-specific and titratable DUX4 expression that shows skeletal muscle disease and this application proposes to develop this into an animal model suitable for studying the role of the DUX4 protein in both skeletal muscle fibers and in the stem cells for skeletal muscle. This work enables studying skeletal muscle pathology due to the DUX4 gene in vivo, and has the potential to enable testing of therapies for FSHD based in inhibiting the DUX4 protein or RNA.

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5. Derivation of multiple PAX7::GFP FSHD-specific human iPSC lines
Gabsang Lee, D.V.M., Ph.D., Johns Hopkins University, Baltimore, Maryland USA
US$ 94,696
8/1/2017 - 7/31/2018 for one year
FSHS-22017-06

Project Summary

Facioscapulohumeral muscular dystrophy (FSHD) is a developmental disorder in which DUX4 expression is not silenced in early myogenic events. A proper model of early human myogenesis could elucidate a new pathogenic mechanism for FSHD. During the last two generous funding supports from the FSH Society (2012 and 2014), we have established multiple FSHD and healthy control human induced pluripotent stem cell lines (hiPSCs). In addition, the Lee lab has developed a novel ‘chemical compound-based’ skeletal muscle differentiation methodology without using overexpression of myogenic transcription factors, animal products, or even recombinant proteins (published in 2016, Choi et al., Cell Reports). This new protocol is relatively fast (~ 30 days) and faithfully follows in vivo myogenesis. For example, our genetic reporter system (MESOGENIN1::eGFP, as a marker for pre-somite stage) shows that over 80% of the differentiating cells are undergoing the somite stage, suggesting that our protocol is indeed mimicking developmental myogenesis.

The results from our previous funding support (2014 funding from the FSH Society) indicate that the SSEA3+ undifferentiated iPSCs, MESOGENIN1::GFP+ somite cells, and even NCAM+/HNK1- myoblasts cells may not be the best cell type to discern the effects of DUX4 and/or FSHD-related transcriptional

discrepancies (please refer to our research progress report in ATTACHMENT IV). In FSHD patients, it is unclear which cell types express DUX4, and it is extremely difficult to find any DUX4 immunoreactive cells in human primary myoblast cultures. A very recent study from the Zammit group shows that Dux4 is transiently expressed during skeletal muscle regeneration, Dux4 maintains Pax7 expression through transcriptional activation of target genes, and Dux4 induces signatures of a stem-cell-like and less-differentiated state. These data lead us to hypothesize that DUX4 can be expressed in PAX7 expressing cells of FSHD iPSCs, or at least PAX7 expressing cells should be the correct cell type to study FSHD molecular pathogenesis.

My group has developed a strategy to generate 'knock-in' PAX7::GFP reporter lines, and we have established multiple PAX7::GFP reporter human iPSCs. We will continue our efforts to generate PAX7::GFP FSHD-hiPSC and control-hiPSC lines (three genotypes for each) to isolate putative skeletal muscle stem/progenitor cells, followed by detailed cellular and molecular analysis.
These approaches can lead to new insights on FSHD disease mechanisms, and the newly developed cell lines can be shared with other research groups for future in vitro and in vivo studies.

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6. DUX4 toxicity: Deciphering necrotic pathways in FSHD
Julie Dumonceaux, Ph.D., University College London, London, United Kingdom
US$142,400
10/01/2017 - 03/31/2019 for eighteen months
FSHS-22017-07

Project Summary

FSHD is one of the most common muscular dystrophies and so far there is no curative or preventive treatment. It is characterized by a loss of repressive epigenetic marks within the D4Z4 array, leading to chromatin relaxation and, when associated with a permissive chromosome 4, to the expression of the normally silenced DUX4 protein whose ORF is present in each D4Z4 repeat. DUX4 is a transcription factor resulting in a poison protein through induction of downstream genes, which might play a major role in FSHD onset/progression.

DUX4 is often described as toxic for muscle cells in FSHD but cell loss mechanism driven by DUX4 expression remains largely unknown. In the literature, several articles have already investigated DUX4-dependent cell death mechanisms in vitro and in vivo, but focusing on apoptosis pathways. However, whereas FSHD mainly involves cells dying with necrotic morphology in patients biopsies, the necrotic death pathway has never been investigated. Our goal is to investigate necrotic mechanisms in this pathology.

This work will allow a better understanding of FSHD pathophysiology, may explain the link between DUX4 expression and FSHD pathophysiology and may help to define new therapeutic targets.

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7. Transcriptional and epigenetic regulation of D4Z4 at chromosome 4q35.2
Sanxiong Liu, Ph.D., New York University School of Medicine, New York, New York USA
Danny Reinberg, Ph.D., New York University School of Medicine, New York, New York USA
US$125,000

08/01/2017 – 07/31/2018 for one year
FSHS-22017-08

Project Summary

Facioscapulohumeral muscular dystrophy (FSHD) is a currently untreatable genetic disease whereby patients suffer from progressive muscle weakness. The genetic alteration causing FSHD has been mapped to the D4Z4 macrosatellite repeats at chromosome 4q35.2. In healthy individuals, this region has 11-100

copies of D4Z4 and remains intrinsically silenced. However, in FSHD patients, D4Z4 repeats were contracted to be less than 11 copies, leading to epigenetic de-repression of D4Z4 and transcriptional activation of double homeobox 4 (DUX4), a transcription factor residing within D4Z4. Although these phenomena have been well-documented, the factors and mechanisms contributing to DUX4/D4Z4 misregulation still remain largely unknown. Recently, Dux4 was shown to possess transcriptional transactivities and positively regulate germline development and immune response genes that potentially contribute to FSHD pathogenesis. However, upon Dux4 over-expression in myoblasts, a subset of Dux4 direct target genes were found downregulated, underscoring the possibility that Dux4 may participate in functionally distinct complexes to regulate gene expression. To probe the factors regulating DUX4/D4Z4 and dissect the functions of Dux4, we propose to reconstruct the epigenetic landscape of the D4Z4 locus, screen for transcription factors that activate DUX4 expression, and map the interactome of Dux4 in order to uncover the potential regulators of D4Z4 repression, DUX4 expression, and Dux4 function on chromatin, respectively. Specifically, proteins bound to the D4Z4 locus will be purified and identified using proteomics of isolated segmented chromatin (PICh). Candidate transcription factors that potentially regulate DUX4 expression will be screened using a focused shRNA library. Dux4-interactors will be identified by chromatin immunoprecipitation or chromatography fractionation of DUX4 protein followed by mass spectrometry analyses. Functional validation following these target discovery approaches will be performed in normal and FSHD skeletal myotubes as well as patient biopsies. Collectively, these attempts will elucidate the molecular mechanisms underlying FSHD pathogenesis and reveal potential targets for therapeutic interventions to treat FSHD.

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