Analysis of FHOD3 Heterodimers and Isoform ratios

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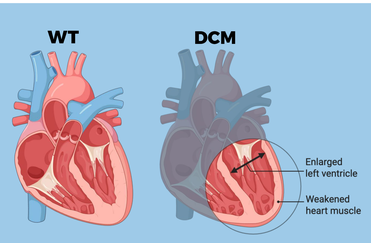
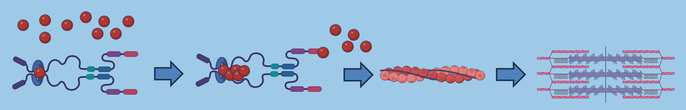
Abstract

Formin Homology 2 Domain-containing 3 (FHOD3) is a highly expressed gene within heart muscle where it is responsible for actin filament elongation and formation of the cardiac sarcomere. The FHOD3 gene transcript is processed into two isoforms: FHOD3S and FHOD3L. The FHOD3L isoform is an essential cardiac protein and contains an exon that promotes its localization to the myocardial sarcomere. FHOD3S lacks such an exon. Typically, FHOD3L and FHOD3S proteins would homodimerize, activating each isoform's ability to function. This research examines the expression ratio between the FHOD3 isoforms in human dilated cardiomyopathy (DCM) tissue and tests whether FHOD3L and FHOD3S isoforms heterodimerize in cellulo. To test for FHOD3 isoform expression ratios, sections of DCM human hearts were processed and run on a western blot. Heterodimerization was tested utilizing a HaloTag affinity pulldown where I constructed a FHOD3L-HALO tagged plasmid. In the pulldown experiment FHOD3L-HALO was the “bait” and would covalently bind to the beads while the untagged FHOD3S was the “prey” and would only be seen in the elution if it dimerized with FHOD3L. We present evidence demonstrating that FHOD3 total is upregulated in DCM tissue (p = 0.0064) relative to control hearts. In addition, we found that FHOD3L is upregulated relative to FHOD3S in the DCM samples (p = 0.0281). Furthermore, western blot results from the HaloTag pulldown suggest that FHOD3L and FHOD3S isoforms heterodimerize in cellulo. Since FHOD3S lacks the sarcomere localization sequence, heterodimerization could pull functional FHOD3L away from the sarcomere. This could establish FHOD3S as a sequestering partner to FHOD3L. Next steps for this project are to study the FHOD3 heterodimers to understand their function and impact on cardiomyocytes.

Introduction



- **DCM is a condition where the left ventricle loses muscle mass making it more difficult to pump blood to the body**

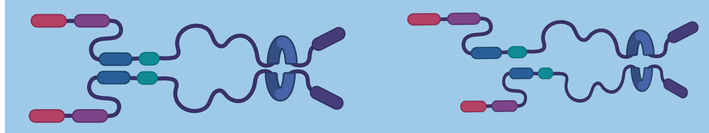


- **Actin assembly of the cardiac sarcomere is facilitated by FHOD3 proteins**



- **FHOD3 is processed into two isoforms FHOD3L which localizes to the sarcomere due to an extra exon and FHOD3S which is expressed in the infant heart**

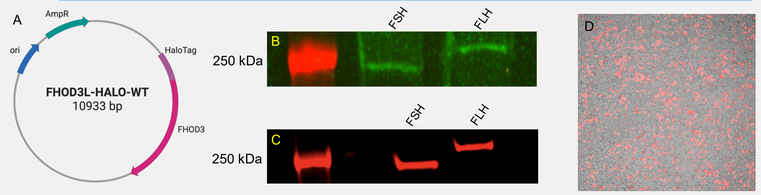
FHOD3 Homodimer **FHOD3 Heterodimer**



- **Different expression ratios of FHOD3 proteins and the formation heterodimers may impact the manifestation of cardiomyopathies**

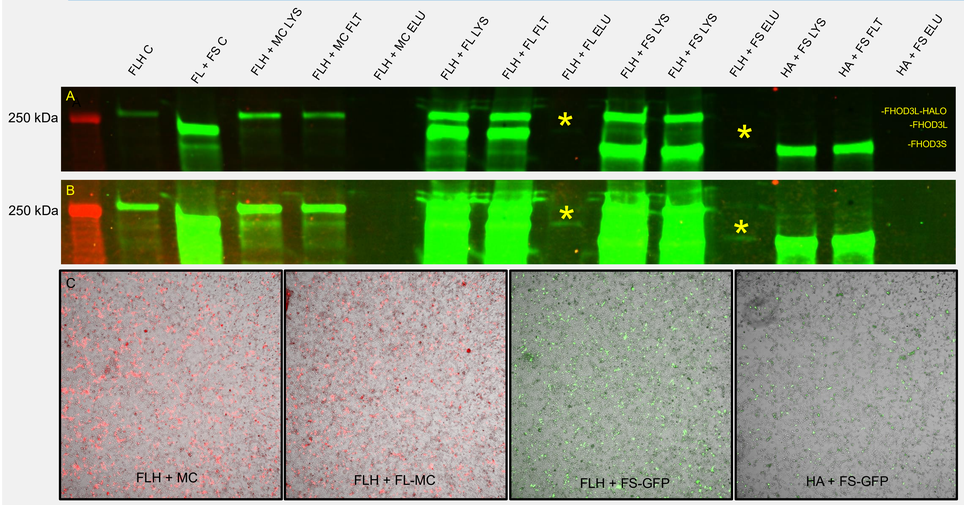
Results

Figure 1. Verification of FHOD3L-HALO construct



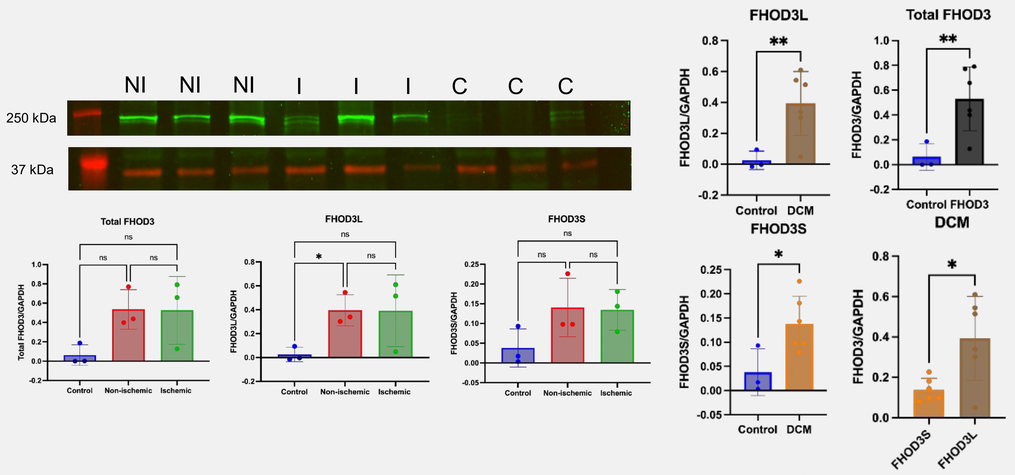
(A) Diagram of FHOD3L-Halo Tag Plasmid. **(B)** FHOD3S-HALO (FSH) and FHOD3L-HALO (FLH) transfected HEK293Tcell lysates were run on a western blot stained with Rabbit-FHOD3 Antibody. **(C)** FHOD3S-HALO (FSH) and FHOD3L-HALO (FLH) transfected HEK293Tcell lysates were run on a western blot stained with Mouse-Myc Antibody. **(D)** Janelia Fluor® 549 HaloTag® Ligand was added to FHOD3L-HALO transfected HEK293T cells in a 24 well plate per manufactures instruction. Red Fluorescent cells are indicative of a functional HaloTag.

Figure 2. Detection of FHOD3L-FHOD3S heterodimers via western blot post HaloLink Pulldown.



(A) Detection of FHOD3L-FHOD3S heterodimers via western blot post HaloLink Pulldown. **(B)** Same western blot image as (A) but over saturated. Results suggest that FHOD3L FHOD3S Heterodimers form due to the presence of the FHOD3S band (yellow asterisk shows appearance of band) in the FLH+FS ELU lane. **(C)** Images of transfected cells. Abbreviations: Lysate (Lys), Flow Trough (FLT), Elution (ELU), FHOD3L-HALO (FLH), Mcherry (MC), FHOD3L-Mcherry (FLMC), FHOD3S-GFP (FS-GFP), Halo Alone (HA). Yellow asterisk indicates eluted proteins from HALO pulldown.

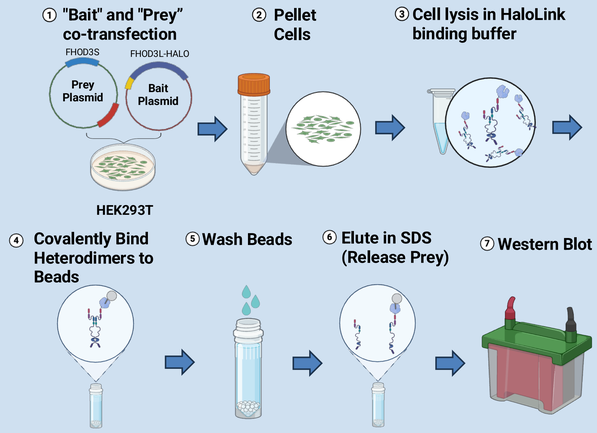
Figure 3. DCM hearts upregulate expression of FHOD3 relative to control hearts and upregulate FHOD3L relative to FHOD3S



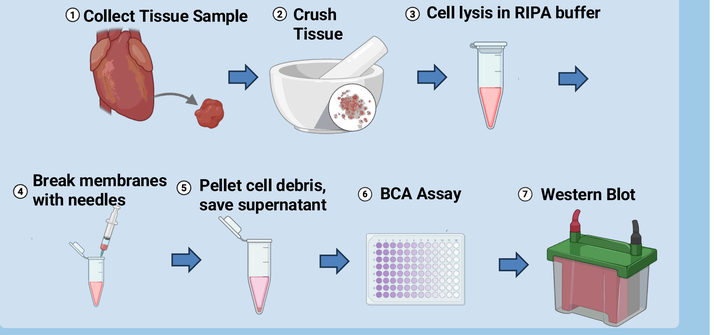
(A) Western blot of DCM non-ischemic (NI), DCM ischemic (I), and control (C) human hearts stained with FHOD3 antibody and GAPDH. Samples were processed following the dilated cardiomyopathy FHOD3 isoform expression protocol detailed in the methods section. **(B)** Graphs comparing isoform expression ratios in total DCM samples compared to control. **(C)** Graphs comparing total isoform expression ratios between non-ischemic, ischemic, and control hearts. Results show that FHOD3L is upregulated in non-ischemic DCM hearts relative to control hearts (p=0.037), that total FHOD3 is upregulated in DCM hearts relative to control hearts (p=0.0064), that FHOD3L is upregulated in DCM hearts relative to FHOD3L in control hearts (p=0.0061), that FHOD3S is upregulated in DCM hearts relative to FHOD3S in control hearts (p=0.0422), and that FHOD3L is upregulated relative to FHOD3S in DCM Hearts (P=0.0281)

Methods

Heterodimerization HaloLink Pulldown



Dilated Cardiomyopathy FHOD3 isoform expression



Conclusions

- **FHOD3L is upregulated in non-ischemic DCM hearts (p=0.037)**
- **FHOD3 Total is upregulated in DCM hearts (p=0.0064)**
- **FHOD3L is upregulated in DCM hearts (p=0.0061)**
- **FHOD3S is upregulated in DCM hearts (p=0.0422)**
- **FHOD3L is upregulated relative to FHOD3S in DCM Hearts (P=0.0281)**
- **Western blot suggests FHOD3L-S Heterodimers form**

Acknowledgements

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