Fggy Carbohydrate Kinase Domain Containing is Induced During Skeletal Muscle Atrophy and Regulates MAP Kinase Signaling

Anastasia Smith, Mahnoor Izhar, Erisa Joka, and David Waddell, Ph.D.
University of North Florida, Department of Biology, Jacksonville, FL 32224

Abstract

Skeletal muscle atrophy can result from a range of physiological conditions, including denervation. To better characterize the molecular genetic events of atrophy, skeletal muscle was isolated from mice following 3 and 14 days of denervation. The gene expression profile of the denervated muscle tissue was analyzed by microarray and compared to control muscle to identify novel atrophy-induced genes. The microarray revealed that Fggy carbohydrate kinase domain containing (Fggy) is expressed in skeletal muscle and is induced in response to denervation. Bioinformatic analysis of the Fggy gene locus revealed two validated alternative isoforms, which we have termed Fggy-001 and Fggy-003, which have distinct transcription initiation sites. In order to confirm that Fggy is expressed in muscle cells, the cDNA of the two validated alternative variants was cloned from mouse myoblast cells. Interestingly, a novel alternative splice variant for each of the validated alternative isoforms was also cloned from mouse muscle cells, suggesting at least four Fggy splice variants to be expressed in skeletal muscle. Quantitative RT-PCR (RT-qPCR) was performed using RNA isolated from muscle cells and primer pairs that distinguish the four alternative Fggy transcripts. The RT-qPCR data reveals that all four Fggy transcripts are more highly expressed in differentiated myotubes, compared to proliferating myoblasts. Additionally, ectopic expression of Fggy-001 and Fggy-003 resulted in inhibition of muscle cell differentiation and attenuation of the MAP kinase signaling pathway. Finally, confocal fluorescent microscopy analysis revealed that the Fggy-001 transcripts appear to localize to the cytoplasm, while the Fggy-003 transcripts produce a more punctuate localization pattern throughout the cytoplasm of proliferating muscle cells. The characterization of novel genes that are activated during neurogenic atrophy helps improve our understanding of the molecular and cellular events that lead to muscle atrophy and could eventually lead to new therapeutic targets for the treatment of muscle wasting.

Introduction

Skeletal muscle atrophy results in muscle weakening through the decrease in cross sectional areas of myofibril as protein degradation surpasses protein synthesis (1). Many genes are involved in the intricate pathway that results in muscle wasting. In order to understand what occurs during skeletal muscle wasting, Bodine et al conducted a study which induced muscle atrophy in rats (1). They found two genes to be significantly upregulated in all models of muscle atrophy: MuRF1 and MAFbx (1). MuRF1 and MAFbx were both identified as ubiquitin E3 ligases that are believed to tag specific proteins for degradation or modification. To better characterize skeletal muscle wasting, Furlow et al conducted a microarray analysis of whole genome expression of the gastrocnemius muscle isolated from wild-type mice and MuRF1 knockout mice following 3 days and 14 days of denervation (2). They found that many genes were differentially expressed under atrophy conditions, including a carbohydrate kinase referred to as Fggy (4). The research presented here is an extension of the microarray study conducted by Furlow et al on the Fggy gene, which shows the first evidence that Fggy is expressed in skeletal muscle cells.

Results

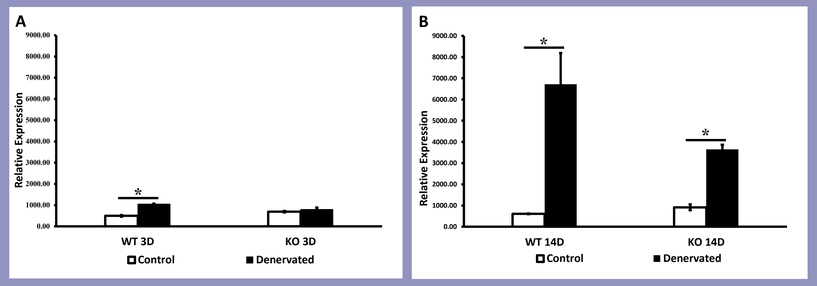

Figure 1. Fggy is induced in response to denervation-induced skeletal muscle atrophy. Whole genome expression analysis was conducted on gastrocnemius muscle from MuRF1-null (KO) and wild-type (WT) mice after **(A)** 3 days (3D) and **(B)** 14 days (14D) of denervation. Significant increase following denervation in wild-type and MuRF1-KO mice (* $p<0.01$) is seen at 14 days post-denervation.

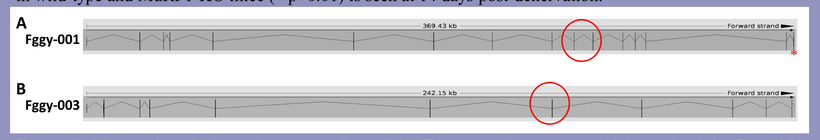

Figure 2. Schematics of alternative transcripts of the Fggy gene in mouse. (A) Schematic of the Fggy-001 transcript and the location of the microarray probe (red asterisk). Fggy-001 has 16 exons and 15 introns. The Fggy-001 alternative transcript is missing exons 10 and 11 (red circle Figure 2A). **(B)** Schematic of the Fggy-003 transcript. The microarray probe did not recognize the Fggy-003 transcript. Fggy-003 has 11 exons and 10 introns. The Fggy-003 alternative transcript is missing exon 7 (red circle Figure 2B).

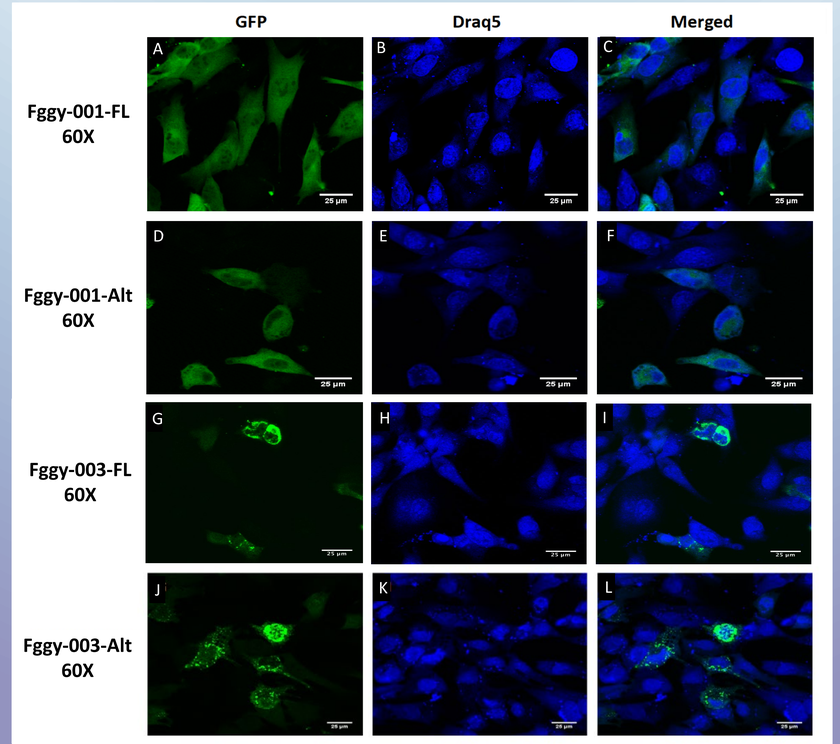

Figure 3. Confocal microscopy of the Fggy-001 FL, Fggy-001 Alt, Fggy-003-FL, and Fggy-003 Alt transcripts fused to GFP in muscle cells. (A-C) Fggy-001-FL shows expression in both the cytoplasm and the nucleus. **(D-F)** Fggy-001-Alt shows expression only in the cytoplasm and appears to be excluded from the nucleus, suggesting that exon 10 and/or 11 may have a nuclear localization signal. **(G-I)** Fggy-003-FL shows expression only in the cytoplasm. **(J-L)** Fggy-003-Alt shows punctate cytoplasmic localization.

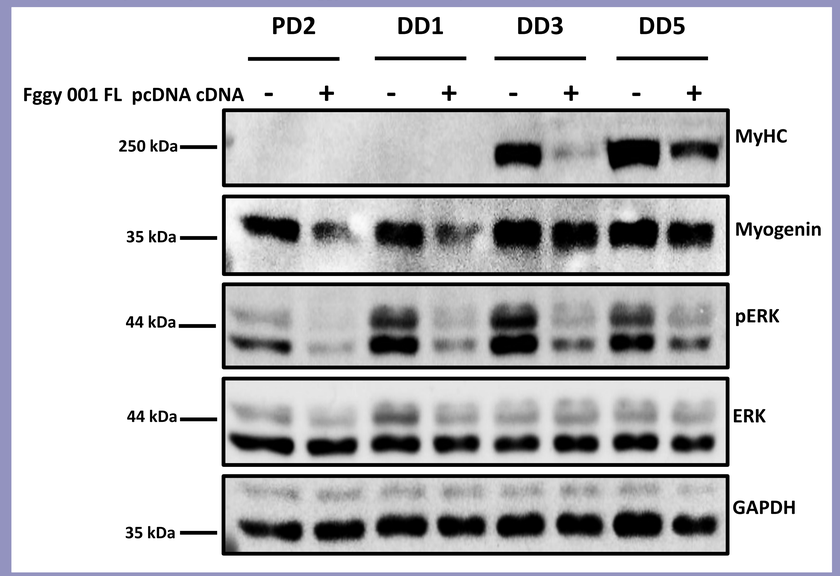

Figure 4. Western Blot analysis of Fggy-001-FL protein over expression probed for differentiation markers and MAPK signaling pathway intermediates. Western blot analysis was performed on proliferating (P) and differentiated (D) C_2C_{12} cells harvested over a ten day time course. Cells were maintained in proliferation media (10% serum) and harvested 2 days post-plating. Cells were then switched to differentiation media (2% serum) and harvested at 1, 3, and 5 days post-media change. The western blot suggests that Fggy overexpression results in an inhibition of differentiation and phosphorylation of ERK.

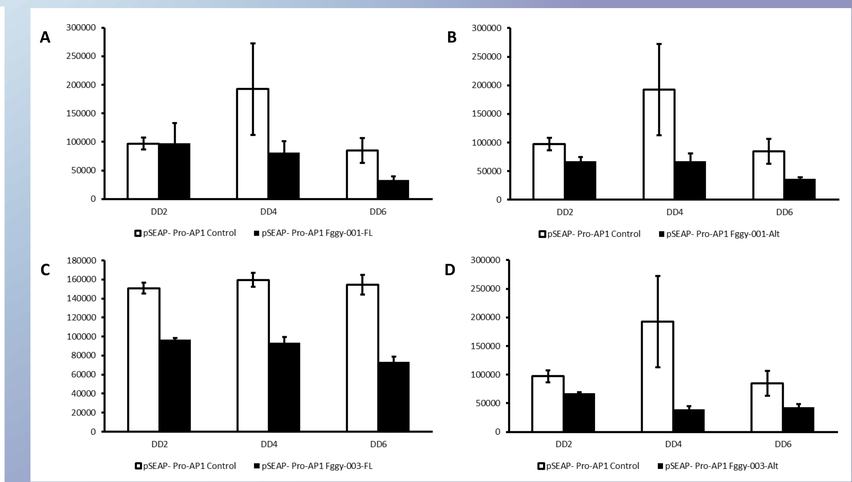

Figure 5. Fggy overexpression attenuates the MAPK signaling pathway. C_2C_{12} cells were transfected with either a reporter gene with an AP-1 consensus element alone (white bar) or in combination with 1 ug of the Fggy pcDNA cDNA expression plasmid (black bar) and culture media was analyzed over a 6-day time-course. Cells were maintained in proliferation media (10% serum) 2 days post-plating and then switched to differentiation media (2% serum).

Conclusion

- Fggy expression was induced in MuRF1-null and wild-type mice following denervation-induced atrophy.
- Fggy expression increased significantly in wild-type mice following 3 and 14 days of denervation, while MuRF1-null mice showed no significant change in expression at 3 and 14 days post-denervation (Figure 1).
- Two transcripts of the Fggy gene were identified and cloned from mouse muscle cells along with novel isoform of each transcript (Figure 2).
- All four transcripts were tagged with GFP to visualize the cellular localization of Fggy in murine muscle cells. It was found that Fggy exhibited nuclear exclusion in all transcripts except Fggy-001-FL (Figure 3).
- Western Blot analysis suggests that the Fggy-001-FL transcript overexpression inhibits C_2C_{12} differentiation when probed for Myosin Heavy Chain and myogenin which are established markers of muscle cell differentiation. Western blot analysis also unveiled a decrease in ERK phosphorylation (Figure 4).
- The role of the ERK protein in the Mitogen Activated Protein Kinase (MAPK) cascade and the effect that the overexpression of Fggy had on it, foreshadowed the results of the AP-1 reporter assays conducted with all four transcripts. The overexpression of Fggy led to a decrease in AP-1 reporter activity in all transcripts, most significantly at the later differentiation time points, when compared to control (Figure 5).

References

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