4-29-2021

Differential Myokine Expression in a Model for Myotonic Dystrophy Type I

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Introduction

Myotonic dystrophy type 1 (DM1) is a multi-systemic disease resulting in severe muscle weakening and wasting. Expanded CUG RNA expression in the DMPK 3' untranslated region leads to the DM1 muscle wasting phenotype though it is unclear the direct mechanisms that generate muscle wasting.

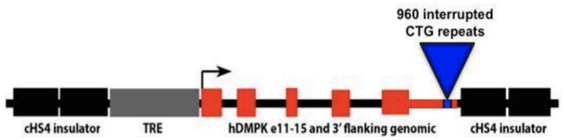


Figure 1: In a previous study (Morriss, et al.), a mouse line was generated containing a tet-inducible transgene (TREDT960I) that contained 960 CTG repeats in the context of human DMPK exons 11-15.

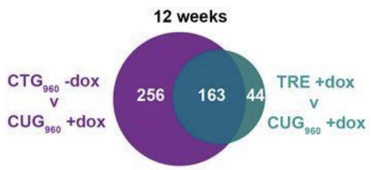


Figure 2: RNA-seq from this previous study revealed broad changes to gene expression in CUG960 +dox mice. Gene expression changes between control (CUG960 – dox or TRE +dox) and repeat-expressing (CUG960 + dox) mice at 12 weeks are displayed. Events changed in CUG960 +dox mice relative to both controls were considered for additional analysis.

Objective

The overall goal of this project is to determine the contribution of altered myokine expression to muscle wasting in DM1. For this semester, we focused on optimization of our previously designed primers customary for myokines that display significant differential expression at 6, 12, and 20 weeks of repeat expression including the *Cx3cl*, *Cxcl10*, and *Gdf5* genes found in previous studies.

Methodology

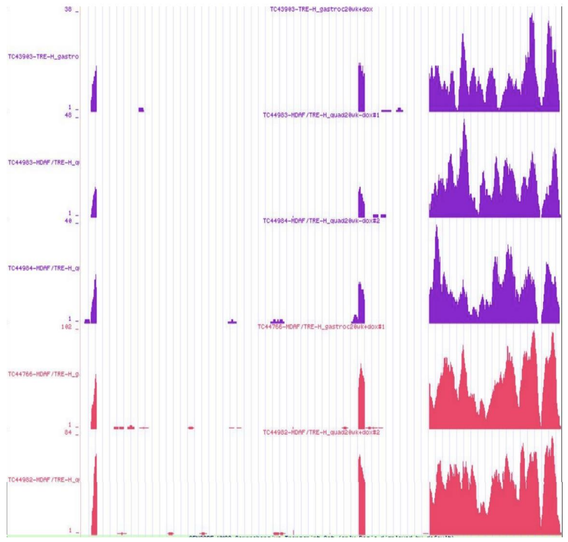


Figure 3: RNA-seq data for the *Cx3cl* gene at 20 weeks based on custom tracks found in a previous study.

These RNA-seq tracks were used to view exons that are displayed in all isoforms. Above the tracks, I was able to find the location of the desired gene and its sequence to plug into the Primer BLAST tool to design primers based on these sequences.

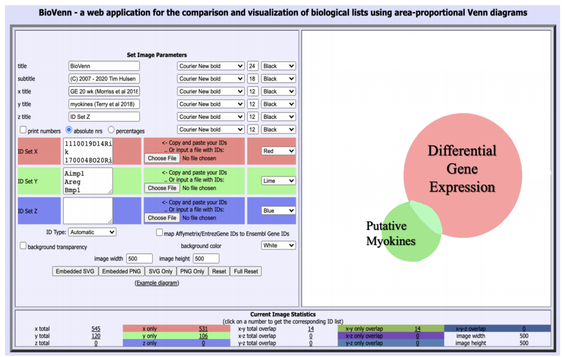


Figure 4: Using BioVenn, a comparison of RNA-seq data between two previous studies, one focusing on putative myokines that were expressed in skeletal muscle (green) and the other representing differential gene expression during muscle wasting (pink), was used to find differentially regulated genes that overlapped in a 20-week period to find our targets.

Gene symbol	Gene name	Data set found
Cx3cl1	chemokine (C-X3-C motif) ligand 1	6, 12, and 20 weeks
Cxcl10	chemokine (C-X-C motif) ligand 10	12 and 20 weeks
Gdf5	growth differentiation favtor 5	12 and 20 weeks
Ccl2	chemokine (C-C motif) ligand 2	20 weeks
Ccl22	chemokine (C-C motif) ligand 22	20 weeks
Ccl5	chemokine (C-C motif) ligand 5	20 weeks
Ccl8	chemokine (C-C motif) ligand 8	20 weeks
Ccl6	chemokine (C-C motif) ligand 6	20 weeks
Cxcl9	chemokine (C-X-C motif) ligand 9	20 weeks
Il15	interleukin 15	20 weeks
Ccl12	chemoline (C-C motif) ligand 12	20 weeks
Il12b	interleukin 12b	20 weeks
Nog	noggin	20 weeks
Tnfsf10	tumor necrosis factor (ligand) superfamily, member 10	20 weeks

Figure 5: Based on this comparison, 14 genes were found to overlap at 20 weeks

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTACTAGGAGCTGCGACACG	Plus	20	27	46	59.97	60.00	6.00	2.00
Reverse primer	AAGCCACTGGGATTCGTGAG	Minus	20	5917	5898	60.04	55.00	5.00	0.00
Product length	5891								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CCGCGTTCTTCCATTTGTGT	Plus	20	123	142	59.41	50.00	4.00	0.00
Reverse primer	TCGTGAGGTCATCTTGTCGC	Minus	20	5904	5885	60.11	55.00	4.00	2.00
Product length	5782								

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCCGCGTTCTTCCATTTGTG	Plus	20	122	141	60.73	55.00	4.00	0.00
Reverse primer	ATAGCGGATGAGCAAAGCCA	Minus	20	5931	5912	59.82	50.00	2.00	1.00
Product length	5810								

Figure 6: These primer pairs were designed for the Cx3cl3 gene.

Primers were designed complementary to each putative myokine through the NCBI tool Primer BLAST. Each myokine was designated 3 suitable primers for variation. Specified regions with constitutive exons were chosen to allow consideration of overall expression levels. These primer pairs are for the Cx3cl3 gene.

Conclusion and Future Works

We found 14 putative myokines that show differential expression in the repeat expressing mice and designed primers for those myokines that will continue to be optimized. The results from the data suggest optimization of the experimental primer designed for the *Cx3cl* gene as well as internal controls for the *Rpl41* and *Actb* genes. The developed primers will be used to validate differential expression levels of these myokines using quantitative RT-PCR. Validated myokines will be assessed in unaffected and DM1 human myoblast cell lines to determine the contribution of these myokines to the skeletal muscle phenotype in DM1.

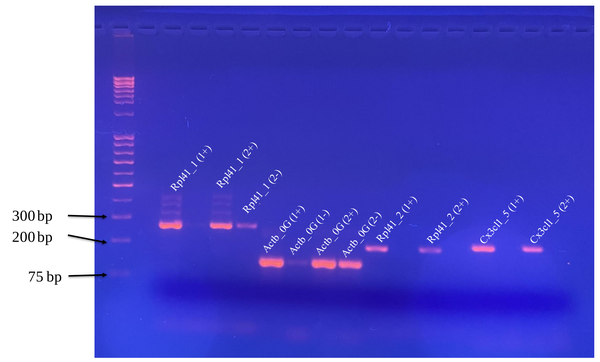
References

- Morriss, G. et al. (2018) 'Mechanisms of skeletal muscle wasting in a mouse model for myotonic dystrophy type 1', Human Molecular Genetics, vol.27, no. 16, pp. 2789 – 2804.
- Terry, E. et al. (2018) ' Transcriptional profiling reveals extraordinary diversity among skeletal muscle tissues', eLife.

Acknowledgements

We would like to thank Dr. April Wynn for allowing us to use her resources to conduct our experiments.

Results



RT-PCR detection for two primer sets designed for the *Rpl41* gene, one for the *Actb* gene, and one for the *Cx3cl* gene. 1+ and 2+ represent two samples of cDNA used for each primer set.

Figure 7: 2% agarose gel composed of 2 g agarose in 100 mL 1x TAE (Tris-Acetate-EDTA). The dyed PCR products were then loaded into the gel and run in an electrophoresis chamber at 100V for approximately 1 hour in a 1X TAE buffer solution. A Q5 Hot Start High-Fidelity 2X Master Mix was used. Denaturation began at 98°C, annealing at 60°C, and extension at 72°C for 35 cycles.