Research Proposal:

Understanding Muscle Atrophy through DGE Analysis

BINF 5005

Integrative Research Project

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Context

250,000 to 500,000

People suffer from SCI each year

20% to 55%

Of muscle atrophy cases appear 6mo-1yr after complete SCI

20% to 30%

Of muscle atrophy cases appear 6mo-1yr after incomplete SCI

Because of the importance of skeletal muscle, **muscle atrophy** can lead to increased risk in:

- Risk of low trauma fractures
 - Risk of osteoporosis
 - Risk of diabetes
- Risk of cardiovascular diseases

What We Know – Effects of SCI

- SCI affects somatic and visceral nerve functions
- Somatic nerve function damage causes denervation of corresponding skeletal muscle (B)
- Motor neurons innervating skeletal muscle undergo apoptosis, leading to motor dysfunction (C)
- Endplates degenerate, acetylcholinesterase content in synapses decreases (D)

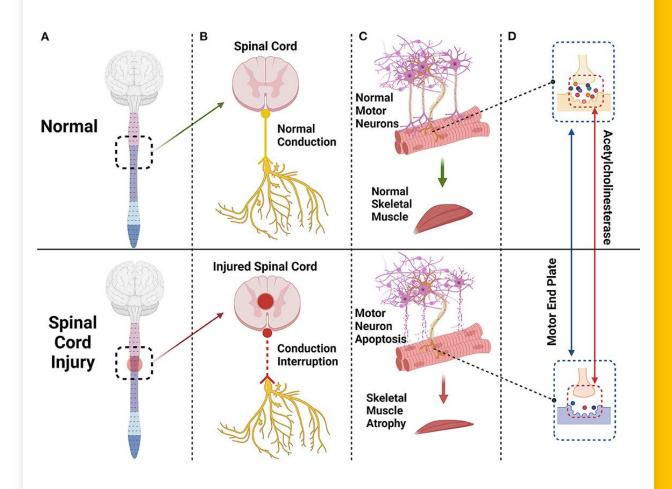


Figure 1: Spinal Cord Injury Effects on Central Nervous System

What We Know – Pathological Changes

Visceral nerve function damage leads to

- disruption of hormone secretion (testosterone, insulin, growth factor)
- Fat deposition
- Protein degradation
- Insulin resistance

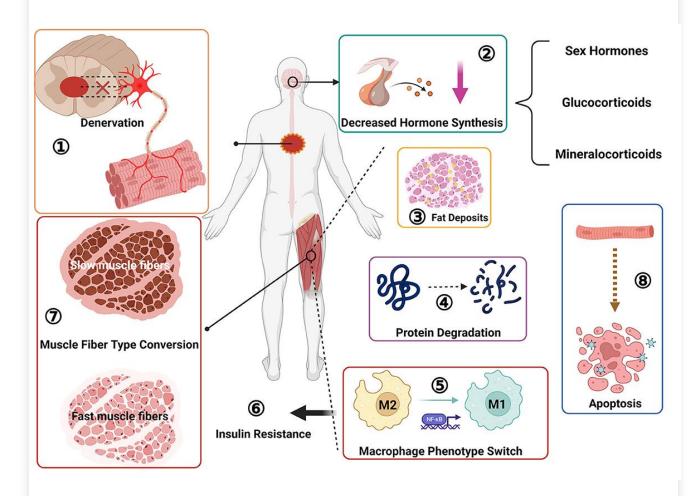


Figure 2: Pathological Changes of Skeletal Muscle after SCI

Current Literature







STUDIES SHOW THAT CHANGES IN PROTEIN
UBIQUITINATION AND ENERGY GENERATION
PATHWAYS AFFECT THE PROCESS OF MUSCLE ATROPHY

DIFFERENT GROWTH FACTORS MAY PLAY IMPORTANT ROLES IN MUSCLE ATROPHY

MANY EXPERIMENTAL STUDIES ON SKELETAL MUSCLE
ATROPHY AFTER SCI AIMED AT PREVENTION AND
TREATMENT

Gaps



Few global gene profiling studies on changes in muscle tissue gene expression a few days following SCI



DEG research in nerve injury-related muscle atrophy tends to focus on either change in expression of specific classes of molecules or variation amongst individual types of nervous injuries

Research Question and Objectives

"How do differentially expressed genes after nerve injuries influence various biological pathways and networks in muscular atrophy?"

- 1. Identify the most differentially expressed genes in our data set
- 2. Analyze pathways that those genes are involved in

Data

- GSE45550 (from GEO Gene Expression Omnibus)
 - Baligand et. al 2015: Study on treadmill locomotor training on rats following surgically-induced SCI
 - **Injury:** dropping a 10g cylinder on exposed spinal cord, causing injury
 - Six groups studied:
 - 1 x Control group
 - 3 x SCI groups (3, 8, and 14 days after SCI)
 - 2 x SCI + locomotor training groups (8 and 14 days after SCI)



Figure 3: Example of treadmill training on Sprague-Dawley rats

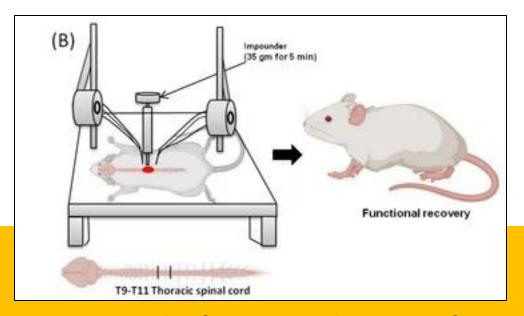


Figure 4: Example of experimental apparatus for SCI

Data (cont.)

- Biopsies of left soleus muscle
 - Samples weighed and mass compared to control
- Affymetrix microarray data
 - Quantitative data on RNA transcripts in samples



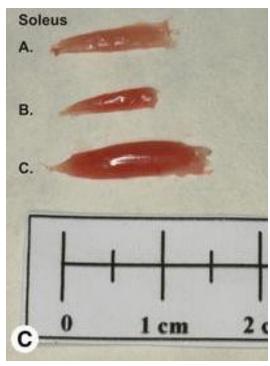


Figure 5: Soleus muscle biopsies

Methods for Analysis

- limma: Linear Models for Mircoarray Data
 - Popular package for analyzing microarray data
 - Fits a linear model & compares experimental to control
 - *t*-statistics and *p*-values
- Differential gene analysis:
 - Find which genes are upregulated or downregulated
- KEGG: analyze the DEGs
 - ShinyGO -> tools for visualizing KEGG connections
- Visualization

DEG Analysis (w/limma) Workflow

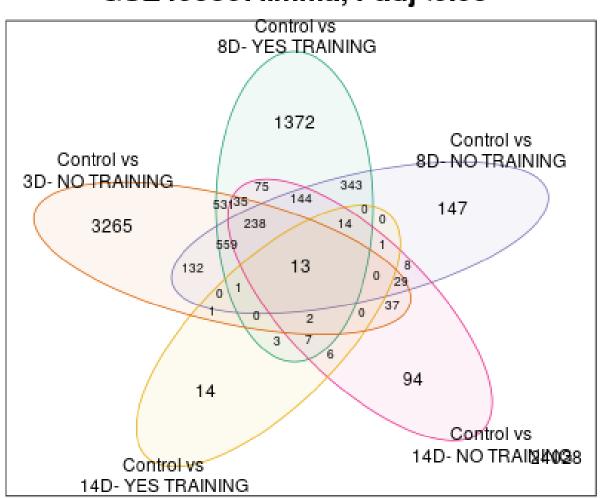
limma analysis **GEO Data Functional** Annotation Affymetrix • DEG • KEGG microarray • **OP:** List of • **OP:** Networks data up/downassociated to regulated • **OP:** .CEL files genes of interest genes

Expected Results

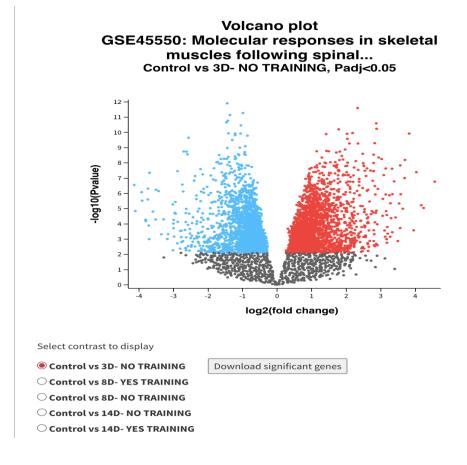
- We analyzed our data (GSE45550) using GEO2R
- Focused on the comparison among 5 groups:
 - 1. CONTROL vs 3D- NO TRAINING
 - 2. CONTROL vs 8D- YES TRAINING
 - 3. CONTROL vs 8D- NO TRAINING
 - 4. CONTROL vs 14D- NO TRAINING
 - 5. CONTROL vs 14D- YES TRAINING

Results: Venn Diagram

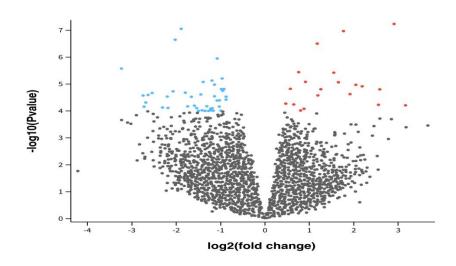
GSE45550: limma, Padj<0.05



Results: Volcano plot



Volcano plot GSE45550: Molecular responses in skeletal muscles following spinal... Control vs 14D- YES TRAINING, Padj<0.05



Select contrast to display

- O Control vs 3D- NO TRAINING
- O Control vs 8D- YES TRAINING
- O Control vs 8D- NO TRAINING
- O Control vs 14D- NO TRAINING

Control vs 14D- YES TRAINING

Download significant genes

Clinical significance

- Muscle atrophy research
 - Help understanding
 - Incorporate gene therapies
- Other tools used on this dataset
 - Wanting to use limma
- Improve quality of life
 - Help with athletic performance
 - Shorten athletes' recoveries
 - Helping with atrophy induced by old-age

Q&A

Thanks for listening!