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Retinol Metabolism and Neuroactive Ligand-Receptor Interactions Are Key Pathways Involved in Skeletal Muscle Stem Cells (MuSCs) Aging

Ryan Ge[1]

[1] Shanghai High School International Division, Shanghai 200231, China
Correspondence: Ryan Ge, Shanghai High School International Division, Shanghai 200231, China.

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Abstract

Sarcopenia is characterized by the loss of muscle function and mass as one ages. Muscle stem cells (MuSCs) play a central role to this process, as it is responsible for muscle repair and regeneration. The progressive loss of function in aging skeletal muscle stem cells is thought to be highly influential. This study aims to analyze RNASeq data obtained from young and old skeletal muscle stem cells of *Mus musculus* and uncover differentially expressed genes between the two groups. These differentially expressed genes were then analyzed to uncover key pathways crucial to the process of MuSC aging. This includes retinol metabolism and neuroactive ligand-receptor interactions.

Keywords: retinol metabolism, neuroactive ligand-receptor interactions, skeletal muscle stem cells, sarcopenia

1. Introduction

At the cellular level, skeletal muscle fibers exhibit a highly organized structure. Each fiber contains numerous myofibrils, which are cylindrical structures composed of repeating sarcomeres, the basic contractile units of muscle (Jing et al., 2022). Sarcomeres consist of overlapping actin and myosin filaments, and their coordinated interaction underlies muscle contraction. Skeletal muscles are innervated by motor neurons, which release acetylcholine at the neuromuscular junction, initiating a series of events that ultimately lead to muscle contraction through the sliding filament theory. This intricate architecture allows for the controlled and finely tuned generation of force and movement.

On the other hand, aging is a process seen in every cellular system, predominantly characterized by a progressive loss of function (Larsson et al, 2019). In skeletal muscles, this is known as sarcopenia, leading to symptoms such as impaired movement and muscle weakness. The role of skeletal muscle stem cells (MuSCs) is highly influential in this process, as it is responsible for the repair and regeneration of skeletal muscle tissue. Loss of MuSC function due to aging also increases the risk of muscular injury. MuSCs undergo a period of postnatal proliferation and differentiation, and enter quiescence once they become adults (Dell'Orso et al, 2019). Located in a niche between the muscle fiber membrane and the basal lamina, these quiescent cells are activated in response to

injury, exercise, or growth stimuli (Brack & Rando, 2012). Upon activation, MuSCs undergo proliferation and differentiation to generate new myocytes, which either fuse with existing muscle fibers or form new fibers altogether. This regenerative capacity is crucial for muscle repair and growth and is essential in maintaining the structural integrity of skeletal muscles throughout an individual's lifespan. Understanding the role of aging in MuSC function is thus of paramount importance in elucidating the mechanisms underlying sarcopenia and aging.

2. Results

2.1 Differential Analysis

To uncover genes that potentially played a role in the loss of MuSC function due to aging, those that were differentially expressed between old and young MuSCs needed to be identified. Hence, differential expression analysis based on gene expression data generated from MuSCs freshly isolated from young (4 months) and old (22 months) male C57BL/6 mice by Benjamin et al (2023) was conducted. This was done using the DESeq2 and tidyverse packages in Bioconductor (code is provided in Supplements).

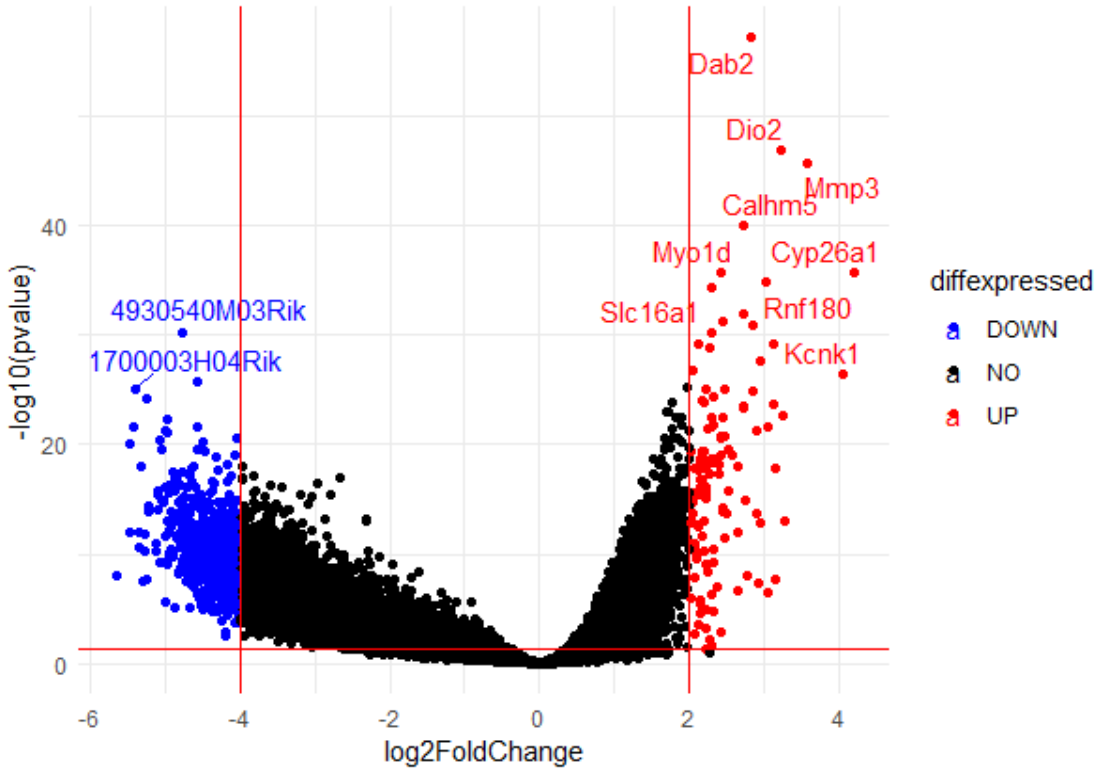

Figure 1. Volcano plot demonstrating differentially expressed genes in old MuSCs relative to young MuSCs

Based on log2FoldChange data and an adjusted-p-value threshold of 0.05, 725 differentially-expressed genes were selected from a database of 24572 total genes (Figure 1). A log2FoldChange threshold of 2 was selected for upregulated genes (marked in red), while a threshold of -4 was selected for downregulated genes (marked in blue). These genes belonged to a variety of pathways, including retinol metabolism, bile secretion, neuroactive ligand-receptor interaction, glutathione metabolism, ECM-receptor interaction, complement and coagulation cascades, steroid hormone biosynthesis, cytochrome p450, and more.

2.2 Enrichment Analysis

To understand the role that these genes played, gene set enrichment analysis using the KEGG pathway database was performed to understand the cellular pathways that these genes participated in and affected. Some of the most prominently featured pathways — that is, those with the highest enrichment scores (Figure 2A) — included retinol metabolism and neuroactive ligand-receptor interactions. Figure 2B is a cnet

plot visualizing the genes involved in these pathways, with the neuroactive ligand-receptor interaction pathway involving up to 25 differentially expressed genes.

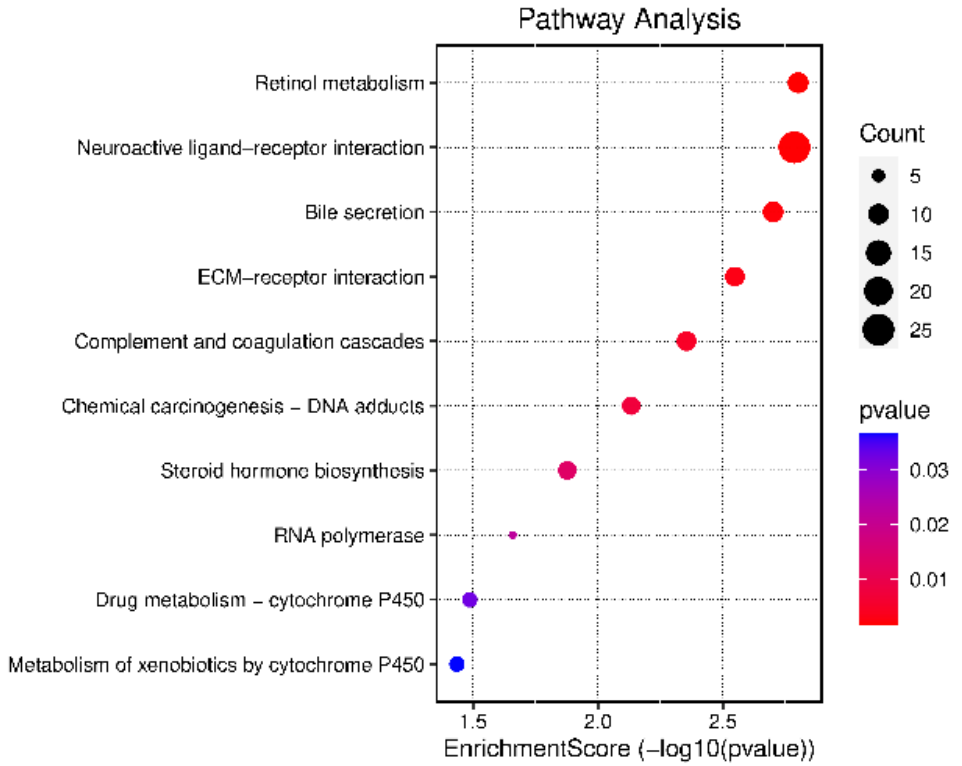

Figure 2A. Enrichment scores, gene counts, and p-values of notable pathways featured in enrichment analysis

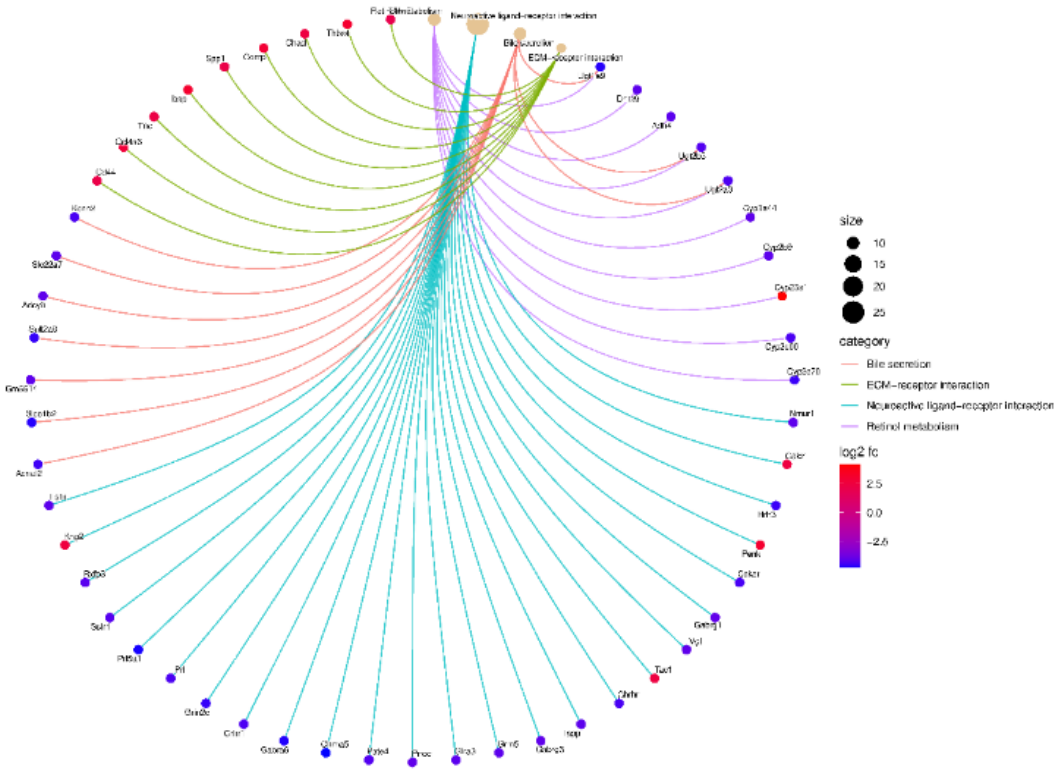

Figure 2B. Cnet plot summarizing and categorizing the pathways, genes, and log2FoldChange data found via enrichment analysis using the KEGG pathway database

2.3 2 Identified Pathways

The retinol metabolism pathway (Figure 3) is known to be a crucial factor in many cellular mechanisms, and MuSCs are no different. Recent studies have suggested that retinoic acid plays an instrumental role in stem cell differentiation, and especially myogenic differentiation in particular (Rönn et al, 2015) (Chen & Li, 2016). Hence, the presence of differentially-expressed genes in the retinol metabolism pathway suggests that as MuSCs age, its ability to respond to retinoic acid may change. This alters its ability to undergo effective differentiation and proliferation. The former is instrumental in processes such as muscle repair and regeneration, while the latter is key in maintaining a healthy population of quiescent MuSCs. Therefore, aging-related differential gene expression may cause a loss of the aforementioned functions. Furthermore, other recent studies in mice have suggested that a diet low in retinol affects skeletal muscle function by reducing glycogen stores, Ca^{2+} transients, and the force exerted by the muscles in question (Ruiz et al, 2021). This shows that retinol plays a crucial role in skeletal muscle function, and that aging-related differential gene expression in this pathway may ultimately contribute to a further loss of function.

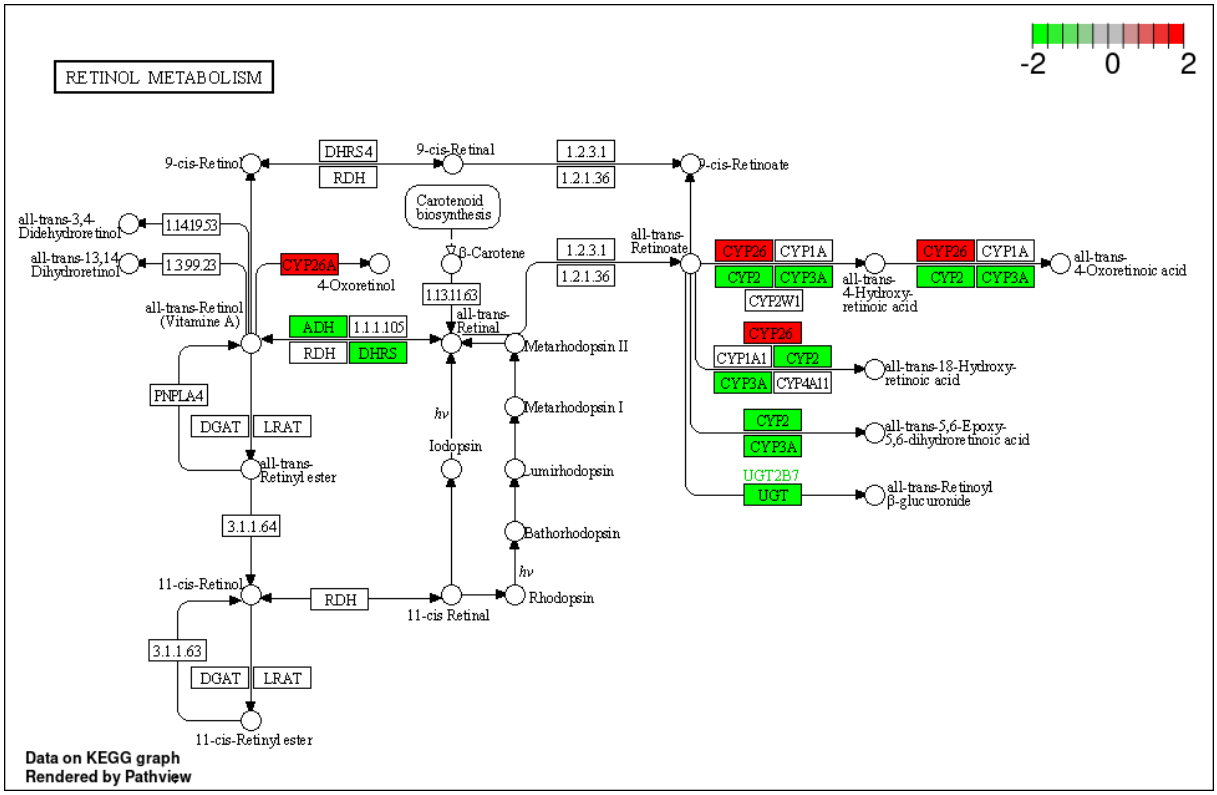

Figure 3. The retinol metabolism pathway and differentially expressed genes in old MuSCs relative to young MuSCs

Neuroactive ligand-receptor interactions (Figure 4) are crucial to the response of cells to external stimuli. In this case, the cells in question would be MuSCs. A high amount of differentially expressed genes from this pathway in older MuSCs compared to younger MuSCs suggest that aging may have altered the function of these ligand-receptor interactions, affecting MuSC response to stimuli such as injury, exercise, or growth. This could possibly contribute to the loss of function and regenerative capacity in aged muscle tissues, which in turn is a highly characteristic feature of sarcopenia.

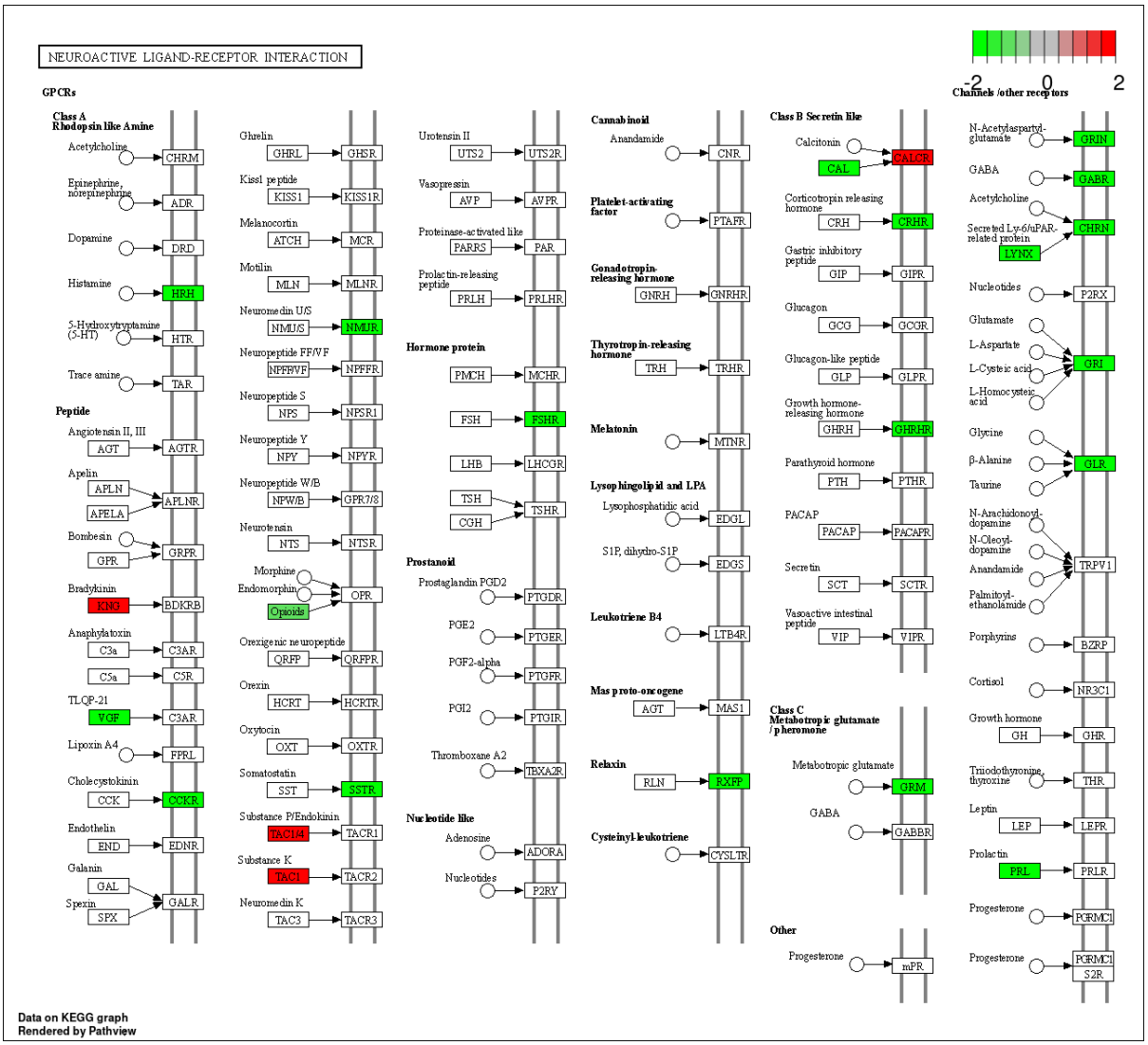

Figure 4. The neuroactive ligand-receptor interaction pathway and differentially expressed genes in old MuSCs relative to young MuSCs

3. Discussion

Differential expression analysis of the 24572 genes from isolated young and old *Mus musculus* MuSCs, followed up by gene pathway enrichment analysis using the KEGG pathway database revealed retinol metabolism and neuroactive ligand-receptor interaction pathways as key candidates for pathways crucial to MuSC function and activity relative to aging. These pathways may be central to the development and progression of sarcopenia, and may prove to be notable points of study in future research. Furthermore, this sheds light on the potential functionality of retinol metabolism and neuroactive ligand-receptor interactions in MuSCs.

In particular, the identification of retinol metabolism as a significant pathway supports recent existing research regarding the role of retinol metabolism in MuSC quiescence. Notably, research by Luo et al (2022) demonstrate that retinol and retinol metabolites maintain MuSC quiescence "through regulation of translation initiation". Retinol metabolism is demonstrated to be enriched in quiescent MuSCs, whereas retinol metabolites such as retinoic acid are found to inhibit MuSC proliferation and differentiation, while similarly inhibiting MyoD protein ssynthesis via the Akt/eIF4EBP1 signaling cascade. Thus, retinol metabolism may play a highly significant role in understanding sarcopenia.

The significant correlations between the age of MuSCs and altered gene expression revealed in

this study, however, does not reveal the exact mechanism by which aging occurs and affects the functionality of MuSCs. Further genetic ablation and genetic rescue studies may shed light on specific functions of the various differentially expressed genes identified above, enhancing understanding of said mechanisms. Another point of interest involves investigation of the upstream or downstream of differentially expressed genes to better understand the mechanisms causing the upregulation or downregulation of the various differentially expressed genes. These mechanisms are not well-understood currently and remain largely unknown.

References

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Supplements

The code used to conduct differential expression analysis in RStudio is shown below. The gene expression data used is from supplemental data provided by Benjamin et al.'s (GEO accession: GSE152793).

```
library(DESeq2)
library(tidyverse)

#load data and col
data <-
read.table("C:/Users/ryang/Desktop/Comps &
Activities/Soft Tissue
Research/GSE152793_Aging_MuSC_RNASeq.txt",
header = TRUE)
col <-
read.table("C:/Users/ryang/Desktop/Comps &
Activities/Soft Tissue Research/col.txt",
header = TRUE)

#process data
data <- data[!duplicated(data$Symbol), ]
rownames(data) <- data$Symbol
data$Symbol <- NULL

#process col
rownames(col) <- col$Sample
col$Sample <- NULL
```

```
#convert to integer
data$O1 <- 1000 * (data$O1)
data$O2 <- 1000 * (data$O2)
data$O3 <- 1000 * (data$O3)
data$O4 <- 1000 * (data$O4)
data$Y1 <- 1000 * (data$Y1)
data$Y2 <- 1000 * (data$Y2)
data$Y3 <- 1000 * (data$Y3)
data$Y4 <- 1000 * (data$Y4)

#check if theres NA
is.na(data)

#create dataframe
dds <- DESeqDataSetFromMatrix(countData =
round(data), colData = col,
                          design = ~ Age)

#reference level
dds$Age <- relevel(dds$Age, ref = "Young")

#perform the test
dds <- DESeq(dds)
#obtain results
res <- results(dds)

#start graphing, get data first
finalres <-
read.csv("C:/Users/ryang/Desktop/Comps &
Activities/Soft Tissue Research/result.csv",
header = TRUE)

#graph, adjust p value, simplify
p <- ggplot(data = finalres,
aes(x=log2FoldChange, y=-log10(pvalue))) +
geom_point() + theme_minimal()
p2 <- p + geom_vline(xintercept=c(-0.6, 0.6),
col="red") +
  geom_hline(yintercept=-log10(0.05),
col="red")

#labelling, subject to change later - especially
the log2foldchange
finalres$diffexpressed <- "NO"
finalres$diffexpressed[finalres$log2FoldChang
e > 2 & finalres$pvalue < 0.05] <- "UP"
finalres$diffexpressed[finalres$log2FoldChang
e < -4 & finalres$pvalue < 0.05] <- "DOWN"

finalres$delabel <- NA
finalres$delabel[finalres$diffexpressed !=
"NO"] <- finalres$X[finalres$diffexpressed !=
"NO"]

#final plot
library(ggrepel)

p <- ggplot(data=finalres,
aes(x=log2FoldChange, y=-log10(pvalue),
col=diffexpressed, label=delabel)) +
  geom_point() +
  theme_minimal() +
  geom_text_repel() +
  scale_color_manual(values=c("blue", "black",
"red")) +
  geom_vline(xintercept=c(-4, 2), col="red") +
  geom_hline(yintercept=-log10(0.05),
col="red")

#take selected genes for GO analysis
selectedgenes <- subset(finalres,
log2FoldChange > 2 | log2FoldChange < -4 & padj
< 0.05)
selectedgenes <-
selectedgenes[,c("X","log2FoldChange")]
colnames(selectedgenes) <- NULL
write.table(selectedgenes,
"C:/Users/ryang/Desktop/Comps &
Activities/Soft Tissue
Research/selectedgenes.txt", sep = "\t", quote
= FALSE, row.names = FALSE)
```