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Research Article

Comparison of Gene Expression Patterns Associated with Myostatin Using Transcriptomic Data of the Myogenic C2C12 Cell Line and Skeletal Muscle Tissue

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Abstract

TGF-β group member myostatin (MSTN) inhibits the growth and differentiation of skeletal muscle. It is unknown how total inhibition of MSTN restricts the hyperproliferation of muscle cells, though. This study aimed to determine the differentially expressed genes and biological pathways associated with myostatin in mouse C2C12 myogenic cells and skeletal muscle tissue, utilizing high-throughput mRNA expression data. In both tissue and cell line, two experimental groups were compared, including wild-type vs. MSTN-Knockout. Transcriptome data were extracted from GEO and analyzed using the LIMMA package in the R environment and GEO2R. Significantly differentially expressed genes were considered as adjusted p-value < 0.05 with log fold change > |0.5|. Lastly, the possible biological pathways were examined with the KEGG database, and the protein-protein interaction (PPI) network was created using Cytoscape software. To find key genes in the PPI network, a topological analysis was conducted using the Network Analyzer tool in Cytoscape software. The total number of differentially expressed genes was 14549 for the C2C12 cell line and 45267 for mouse skeletal muscle tissue; among them, 235 and 1425 transcripts were significant with an adjusted p-value <0.05. The comparison between these two DEG lists showed that 49 genes were common between the myogenic C2C12 cell and skeletal muscle tissue. Additionally, three biological pathways between the C2C12 cell line and the skeletal muscle tissue were in common, and they were associated with the decrease of MSTN gene expression, including: hypertrophic cardiomyopathy, Axon guidance, and dilated cardiomyopathy. These pathways were all related to muscle tissue. Despite the large number of DEGs, only 49 were in common in tissue and cell line; this could indicate that comparing a tissue with its relevant cell line at the transcriptome level might not be precise enough to draw a solid conclusion. This is due to the nature of cell heterogeneity of most tissues, including skeletal muscle.

***Keywords*:** Myostatin, mouse, Gene network, C2C12 cell line, tissue muscle

Introduction

Myostatin (MSTN), often referred to as growth differentiation factor 8, is a protein that controls how animals, including humans, develop their muscles. It belongs to the protein superfamily known as TGF-ß (transforming growth factor-beta) (Soleimani et al., 2019). Skeletal muscle size and strength are tightly regulated by the hormone myostatin. By preventing muscle cell proliferation and differentiation, it serves as a negative regulator of muscle growth (Riasi et al., 2024). Muscular hypertrophy, often known as muscular growth, is a complex biological process controlled by a number of molecules (Guller and Russell, 2010). Myostatin signaling dysregulation has been linked to several muscular illnesses and diseases that cause muscle loss. Myostatin prevents excessive muscle production by inhibiting muscle cell proliferation, which helps keep muscle mass within a reasonable range (Bowen et al., 2015). Myostatin-related muscular hypertrophy is a condition marked by increased muscle mass and strength that can be brought on by mutations or defects in the myostatin gene (Riasi et al., 2022). Both rare genetic mutations in people and specific breeds of cattle, such as Belgian Blue cattle or Texel sheep, have been linked to this abnormal MSTN expression. Compared to the general population, these people have much more developed muscles (Kambadur et al., 1997). Studies using mdx (muscular dystrophy model for x-chromosome) mice, which have a mutation in the dystrophin gene and are used as a genetic model of Duchenne's and Becker's muscular dystrophy, have suggested that myostatin may be relevant to the treatment of disease in humans (Sicinski et al.,

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1989). For instance, it was discovered that mdx mice without myostatin were not only stronger and more muscular than their mdx counterparts with normal myostatin, but also showed less fibrosis and fatty remodeling, indicating enhanced muscle regeneration (Wagner et al., 2002). Additionally, myostatin increases muscle mass and specific force when it is injected into either wild-type or mdx mice, indicating that myostatin is a key player in controlling muscle growth in adult animals (Bogdanovich et al., 2002; Whittemore et al., 2003).

Investigating gene expression is crucial to comprehending how an organism functions at the system level. The development of next-generation sequencing (NGS) technologies like RNA-sequencing (RNA-seq) during the past few years has given researchers essential tools for analyzing changes in transcriptome abundance (Ekblom and Galindo, 2011). Although RNA-Seq is a powerful and cost-effective method for studying gene expression in bulk samples, it cannot resolve individual cell differences. Single-cell RNA-Seq offers a deeper, more granular view of cellular diversity and gene expression, but it comes with higher costs, technical challenges, and data analysis complexity (Lafzi et al., 2018). Bulk tissue RNA-Seq is ideal for understanding gene expression in the context of complex, heterogeneous tissues and real-life biological conditions, but it has limitations in capturing cell-specific variations. Cell line RNA-Seq is more suited for controlled, reproducible studies of specific cellular responses, but the data may not fully represent in vivo gene expression due to the lack of cellular diversity and the potential for culture-induced artifacts. The choice between using bulk tissue RNA-Seq versus cell line RNA-Seq largely depends on the research question and the level of complexity required in the study. Often, a combination of both approaches may provide a more comprehensive understanding of gene expression and its regulation.

This manuscript aims to explore the use of mRNA expression data for the analysis of genes associated with myostatin and their role in muscle growth. Furthermore, we highlighted specific genes and pathways that have been identified through mRNA expression analysis to gain a comprehensive understanding of the molecular mechanisms underlying myostatin-related gene expression in mouse skeletal muscle tissue and the myogenic C2C12 cell line. The similarity and differences of gene expression patterns and pathways in both MSTN-knockout muscle tissue and cell line were compared.

Materials and Methods

The purpose of the present study is to compare the differences and similarities in genes involved in the pathway regulation of myostatin at two different sample types, mouse skeletal muscle tissue and the C2C12 cell line. To examine the genes in the C2C12 cell, the mRNA expression data in the study conducted by Huang et al (2019 obtained from the NCBI (GEO) database was used (GSE124196) (Huang et al., 2019). The data collected for this study belong to two groups of C2C12 mouse cells as control (n=3) and knockout cell line for myostatin (n=3). R software version 4.0.2 and the LIMMA package were used to convert these data into differential gene expression data (DEGs) (Saedi et al., 2022).

Differential expression data from muscle tissue of myostatin knockout and wild type were obtained from the NCBI GEO (GSM1542298) database. The data belonged to two groups of 35-day-old Balb C mice (n=5) and control (n=5). The differentially expressed genes (DEGs) were obtained by the GEO2R online R software tool (Gorji et al., 2019). Mouse Ensemble gene IDs were converted to official gene symbols using the ENSEMBL Symbol Converter tool (https://www.biotools.fr/mouse/ensembl_symbol_converter).

Data selection criteria for all DEGs were considered as adjusted p-value<0.01 and -0.5> logFC >0.5. To construct the protein-protein interaction (PPI) network of significant DEGs, Cytoscape software version 3.7.0 was used with the STRING version 1.5.1 plugin. The Network Analyzer version 2.7 was used to analyze the topology of the PPI network (Miryala, Anbarasu, & Ramaiah, 2018). ClueGO version 2.5.9 and CluePedia version 1.5.9 were used for Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis. Significant KEGG pathways were selected based on Bonferroni corrected p-value ≤0.05 (Javadmanesh and Riasi, 2023).

Results

The complete results of the differential expression of gene analysis in this study are given in the supplementary table 1 (supplementary file). The initial number of differentially expressed genes was 14549 for C2C12 cell lines and 45267 for muscle tissue, which were changed to 235 and 1425, respectively, after filtering by p-value and log fold change. The gene network was drawn with the STRING plugin of the Cytoscape software. There

were 49 common genes between these two data series, which are reported in Table 1. CXCL12, CSRP3, MB, MYH7, MYOZ2, PDLIM3, TNNC1, TNNI1, TNNT1, and LMOD2 exhibited higher scores in both networks among the shared genes (Table 1).

The list of differentially expressed genes was entered into the ClueGo plugin of Cytoscape to find related significant KEGG pathways; 18 pathways for cell lines and 6 pathways for muscle tissue were discovered (Tables 2 and 3). These pathways were further analyzed to show if there is a connection with each other and related genes, forming a unique network consisting of pathways and genes (Supplementary Figures S1 and S2). These two networks shared only three significant KEGG pathways: hypertrophic cardiomyopathy, dilated cardiomyopathy, and Axon guidance. These common pathways were connected to some important genes such as CACNG1, MYH7, Ablim1, Cxcl12, and TNNC1.

Overall, 20 tissue-related genes and 17 cell line-related genes were shared by each of the biological pathways out of the top 40 genes in both networks. The tissue gene network data indicated that the genes PPARA, MGLL, LPL, Scd1, FABP4, PCK1, and Acaa1b were more important genes, with degrees of 27, 12, 17, 16, 18, 11, and 8, respectively. PPARA signaling, pyruvate metabolism, and cardiac muscle contraction pathways all shared the gene PCK1. The top-ranked gene in the network, ACTB, is linked to three pathways: the RAP1 signaling pathway, focal adhesion, and arrhythmogenic right ventricular cardiomyopathy (ARVC). This was discovered by looking at the biological pathways connected to the cell line data. Additionally, the degrees 117, 62, 45, 56, 47, and 28 of the genes ITGB1, ACTG1, ITGB7, ITGB5, ITGB6, and LAMA2 in the network, respectively, were regarded as key genes in the direction of KEGG biological pathways.

Table 1. Common significantly differentially expressed genes (DEGs) with the highest degree in protein-protein interaction (PPI) network of mouse muscle tissue and C2C12 cell line

Genes	The degree in the C2C12 cell line gene network	The degree in the muscle tissue gene network	Genes	The degree in the C2C12 cell line gene network	The degree in the muscle tissue gene network
Cxcl12	90	10	Pstpip2	10	1
Csrp3	56	18	Rgs2	10	2
Pdlim3	53	9	Ephx2	9	5
mb	52	13	Gatm	9	3
Myh7	52	16	Bdh1	8	5
Myoz2	49	18	Csad	8	2
Pdlim7	40	4	Slc16a3	8	3
Tnni1	39	15	Aoc3	7	4
Tnnt1	38	13	Ccdc80	7	1
Mgst1	33	3	Ddc	7	1
Lmod2	32	11	Dkk2	6	3
Pdlim1	32	6	Dnase2a	6	1
Lpl	25	21	Cmbl	5	3
Mybph	23	7	Elovl3	5	7
Ankrd2	22	7	Evc	5	0
Igf2	22	11	Ablim1	4	0
Smyd1	22	6	Sytl2	4	0
Adcy3	20	3	Lrrn1	3	1
Sorbs1	20	4	Rcan2	3	1
Acsl1	16	23	Plekhb2	2	0
Cacng1	13	2	rgcc	2	0
Acss2	11	16	Stbd1	2	1
Ak4	11	1	Slc44a2	1	0
Grb14	11	0	Vash2	1	0
C1ra	10	4			

Table 2. The Kyoto encyclopedia of genes and genomes (KEGG) significant pathways of differentially expressed genes (DEGs) in the C2C12 cell line

Pathways	P-value (corrected with Bonferroni)	Associated Genes
Rap1 signaling pathway	0.01	[Adcy3, Angpt4, Apbb1ip, Arap3, Calml4, Crkl, Csf1, Efna5, Epha2, Fgf10, Fgf21, Fgf7, Fgfr4, Flt1, Hgf, Itgb1, Lpar2, Lpar3, Magi1, Map2k6, Mapk1, Mapk12, Ngfr, Nras, Pdgfb, Pdgfrb, Pgf, Plcb1, Rac3, Rala, Rapgef3, Sipa1l1, Tiam1, Vegfd]
Cytokine-cytokine receptor interaction	0.05	[Amh, Ccl11, Ccl17, Ccl2, Ccl25, Ccl7, Ccl8, Ccl9, Cntfr, Csf1, Csf2rb, Ctf1, Cx3cl1, Cxcl1, Cxcl12, Cxcl13, Cxcl5, Cxcr4, Cxcr6, Eda2r, Flt1, Ghr, Hgf, Il4ra, Il6ra, Inhba, Inhbb, Lif, Ngfr, Pdgfb, Pdgfrb, Ppbp, Tgfb2, Thpo, Tnfrsf1b, Tnfrsf9, Tnfsf12, Vegfd]
PI3K-Akt signaling pathway	0.01	[Angpt4, Ccnd3, Cdkn1a, Col4a5, Col4a6, Col6a1, Col6a2, Csf1, Ddit4, Efna5, Epha2, Fgf10, Fgf21, Fgf7, Fgfr4, Flt1, Ghr, Gng11, Gng8, Gngt2, Hgf, Il4ra, Il6ra, Itgb1, Itgb5, Itgb6, Itgb7, Lama2, Lamb3, Lpar2, Lpar3, Mapk1, Ngfr, Nras, Pdgfb, Pdgfrb, Pgf, Phlpp1, Pik3r5, Pkn3, Rxra, Sgk1, Sgk3, Thbs2, Tlr2, Tnc, Vegfd, Ywhah, Ywhaq]
Axon guidance	0.01	[Ablim1, Cxcl12, Cxcr4, Efna5, Enah, Epha1, Epha2, Epha4, Ephb6, Fyn, Itgb1, L1cam, Lrrc4c, Mapk1, Nfatc4, Nras, Pdk1, Plcg2, Plxnb1, Ppp3ca, Rac3, Rhod, Robo1, Sema3d, Sema4a, Sema4c, Sema4f, Sema5a, Sema6b]
Focal adhesion	0.00	[Cav1, Cav3, Ccnd3, Col4a5, Col4a6, Col6a1, Col6a2, Crkl, Flnb, Flt1, Fyn, Hgf, Itgb1, Itgb5, Itgb6, Itgb7, Lama2, Lamb3, Mapk1, Myl12b, Myl9, Mylk, Mylk2, Mylpf, Parvb, Pdgfb, Pdgfrb, Pgf, Rac3, Thbs2, Tnc, Vav3, Vegfd]
Pathways in cancer	0.04	[Adcy3, Appl1, Ar, Bdkrb1, Cdkn1a, Cebpa, Col4a5, Col4a6, Crkl, Cxcl12, Cxcr4, Dapk2, Dvl3, Fgf10, Fgf21, Fgf7, Fos, Foxo1, Fzd6, Gli3, Gng11, Gng8, Gngt2, Hgf, Itgb1, Jup, Lama2, Lamb3, Lpar2, Lpar3, Mapk1, Mmp2, Nras, Pdgfb, Pdgfrb, Pgf, Plcb1, Plcg2, Rac3, Rala, Rb1, Rxra, Smad2, Tcf7l2, Tgfa, Tgfb2, Traf1, Traf4, Vegfd, Wnt10a, Wnt9a]
Hypertrophic cardiomyopathy (HCM)	0.00	[Actc1, Cacnb1, Cacnb2, Cacng1, Des, Itgb1, Itgb5, Itgb6, Itgb7, Lama2, Myh7, Sgca, Sgcb, Sgcd, Sgcg, Tgfb2, Tnnc1, Tnnt2, Tpm2, Ttn]
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	0.00	[Actn2, Actn3, Cacnb1, Cacnb2, Cacng1, Cdh2, Des, Itgb1, Itgb5, Itgb6, Itgb7, Jup, Lama2, Pkp2, Sgca, Sgcb, Sgcd, Sgcg, Tcf7l2]
Dilated cardiomyopathy	0.00	[Actc1, Adcy3, Cacnb1, Cacnb2, Cacng1, Des, Itgb1, Itgb5, Itgb6, Itgb7, Lama2, Myh7, Sgca, Sgcb, Sgcd, Sgcg, Tgfb2, Tnnc1, Tnnt2, Tpm2, Ttn]

Table 3. The Kyoto encyclopedia of genes and genomes (KEGG) significant pathways of differentially expressed genes (DEGs) in mouse skeletal muscle tissue

Pathways	P-value (corrected with Bonferroni)	Associated Genes
Fatty acid degradation	0.00	Acaa1b, Acaa2, Acadl, Acsl1, Acsl5, Aldh9a1
Regulation of lipolysis in adipocytes	0.01	Adcy3, Fabp4, Gnai1, Mgll, Ptger3
Pyruvate metabolism	0.02	**Acss2, Aldh9a1, Ldhb, Pck1**
PPAR signaling pathway	0.00	Acaa1b, Acadl, Acsl1, Acsl5, Fabp4, Lpl, Pck1, Plin2, Plin5, Ppara, Pparg, Scd1, Sorbs1
Axon guidance	0.03	Ablim1, Camk2a, Cxcl12, Gnai1, Nrp1, Pak1, Sema3a, Wnt4
Fatty acid elongation	0.00	Acaa2, Elovl3, Elovl5, Elovl6, Hacd1
Biosynthesis of unsaturated fatty acids	0.00	Acaa1b, Elovl5, Elovl6, Hacd1, Scd1
cGMP-PKG signaling pathway	0.01	Adcy3, Atp1b1, Atp1b2, Atp1b4, Atp2a2, Gnai1, Myh7, Rgs2, Slc25a5
cAMP signaling pathway	0.00	Adcy3, Atp1b1, Atp1b2, Atp1b4, Atp2a2, Camk2a, Gnai1, Pak1, Ppara, Ptger3
Cardiac muscle contraction	0.00	Atp1b1, Atp1b2, Atp1b4, Atp2a2, Cacng1, Myh7, Myl2, Myl3, Tnnc1, Tpm3
Adrenergic signaling in cardiomyocytes	0.00	Adcy3, Atp1b1, Atp1b2, Atp1b4, Atp2a2, Cacng1, Camk2a, Gnai1, Myh7, Myl2, Myl3, Tnnc1, Tpm3
Thyroid hormone signaling pathway	0.01	Atp1b1, Atp1b2, Atp1b4, Atp2a2, Dio2, Rcan2, Wnt4
Proximal tubule bicarbonate reclamation	0.00	Atp1b1, Atp1b2, Atp1b4, Pck1
Gastric acid secretion	0.01	Adcy3, Atp1b1, Atp1b2, Atp1b4, Camk2a, Gnai1
Carbohydrate digestion and absorption	0.00	Amy1, Atp1b1, Atp1b2, Atp1b4, Slc37a4
Hypertrophic cardiomyopathy (HCM)	0.00	Atp2a2, Cacng1, Myh7, Myl2, Myl3, Tnnc1, Tpm3
Dilated cardiomyopathy	0.00	Adcy3, Atp2a2, Cacng1, Myh7, Myl2, Myl3, Tnnc1, Tpm3
Fatty acid degradation	0.00	Acaa1b, Acaa2, Acadl, Acsl1, Acsl5, Aldh9a1

Discussion

These days, high-throughput sequencing technologies are becoming a potent instrument to first elucidate the genetic pathways underlying a phenotype. Using microarray analysis, previous research has identified genes linked to the hypertrophic muscle seen in myostatin propeptide transgenic mice (Zhao et al., 2009). Muscle growth and development are inhibited by MSTN, a secreted growth factor of the TGF-β superfamily that is mostly expressed in skeletal muscle. An individual with a "double-muscled" phenotype may lack proper MSTN transcription. However, neither excessive muscle growth nor myosarcoma, which entails uncontrolled muscle development, is caused by low expression or absence of MSTN (Huang et al., 2019).

In the subsequent investigation, we attempted to examine the expression profiles of genes associated with myostatin in muscle tissue and cells in order to determine whether or not the anticipated outcomes in cell culture are consistent with what occurs in the tissue. The findings of the gene network analysis indicate that the most significant genes in both networks are CXCL12, CSRP3, MYH7, MB, ANKRD2, MYOZ2, LPL, TNNT1, TNNI1, TNNC1, and LPI. These genes have also been shown in earlier research to be effective in suppressing the expression of the myostatin gene. Among the top transcripts, CXCL12 had the greatest number of

microRNA interactions (nine, in total) found in this analysis. Our findings revealed that both gene pathways' protein-protein interactions are downregulated in tissue and cell lines. Finding microRNAs that target CXCL12 is crucial because it has been shown that the CXCR4 pathway is regularly downregulated in the skeletal muscles of cancer-associated cachecious mice and patients, and that in mice with the syndrome, activation of the CXCL12/CXCR4 pathway prevents muscle wasting (Martinelli et al., 2016). Other studies have also validated the findings from our study. on order to identify shared regulatory networks and biological pathways, Freire and their colleagues analyzed pertinent literature data from global gene expression profiling studies on muscle wasting in cancer cachexia. For the first time, they discover novel putative microRNA-regulated gene networks connected to muscle atrophy in cancer cachexia. According to their findings, there may be a connection between cancer cachexia and muscle atrophy and the microRNA/mRNA interactions miR-27a/FOXO1, miR-27a/MEF2C, miR-27b/CXCL12, miR-27b/MEF2C, miR-140/CXCL12, miR-199a/CAV1, and miR-199a/JUNB (Freire et al., 2019). According to research by Dileepan et al. from 2016, miR-140 transfection reduces Cxcl12 expression and release in human airway smooth muscle cells, which also lowers inflammatory response. Mesenchymal cells produced the chemokine CXCL12 and osteopontin that Maeda (2019) found helped a genetically engineered model mouse with skeletal muscle dystrophy. Additionally, they demonstrated how patients with Duchenne muscular dystrophy can benefit from these characteristics (Maeda, 2019).

The expression of the Ankrd2 gene has also been linked to myostatin gene function. The Ankrd2 gene, a response gene, has been linked to sluggish muscle performance, according to studies. Both skeletal and cardiac muscle express Ankrd2 (Kemp et al., 2000). Ankrd2 also interacts with proteins such as myopalladin, calpain-3, and telethonin in addition to titin (Kojic et al., 2004). Type I skeletal muscle fibers are where Ankrd2 is mainly expressed (Tsukamoto et al., 2002). Intriguingly, denervation of fast muscle (gastrocnemius) enhances Ankrd2 expression, whereas denervation of slow muscle (soleus) decreases Ankrd2 level to below detection limit after 4 weeks (McKoy et al., 2005). After eccentric contraction, Ankrd2 mRNA levels also rise (Barash, 2004). Only the manner of contraction (eccentric vs. isometric) and not stress affected gene expression (Hentzen et al., 2006).

A decrease in the number of type 1 fibers correlated with a decrease in Myh7 expression in the soleus. The muscles of late bovine fetuses lacking myostatin similarly showed diminished MYH7 expression (approximately fivefold) (Cassar-Malek et al., 2007). Despite having slightly decreased mean expression in mice with post-developmental myostatin knockouts, Myh7 and other genes that encode slow isoforms of contractile proteins are consistent with our research (Welle et al., 2009). The Myh7 gene mutation is also frequently linked to hypertrophic cardiomyopathies, according to another research (Ahmad et al., 2005). Both the outcomes of the gene network analysis in our investigation and earlier studies have demonstrated the relationship between the genes MYOZ2 and MB, as well as TNNI1 and MSTN. In a previous study by Mutryn et al (Mutryn et al., 2015) Wooden breast myopathy muscle revealed that fiber type moving from fast- to slow-type fibers, because it in commercial broilers exhibited a large elevation of TNNI1, MB, MYBPC1, and MYOZ2 genes compared to unaffected muscle.

The pathways and related genes are listed in Tables 2 and 3. Through analysis, we were able to pinpoint the signaling pathways for dilated cardiomyopathy, Axon guidance, and hypertrophic cardiomyopathy (HCM), which control the growth and development of muscle tissues. Also discovered were pathways such as the ECM-receptor interaction and focal adhesion pathways, which have all been supported by previous studies (Miao et al., 2015), and are involved in the maintenance of muscle structure and myocytes.

Our results showed a few overlapping DEGs in myogenic C2C12 cells and skeletal muscle tissue when MSTN is knocked down. This could be due to the nature of tissue heterogeneity. Up to now, there is a large amount of research conducted in a variety of cell lines (e.g. different cancer cell lines) to suggest biological markers or to find candidate genes involved in disorders or favorable traits (Saliani et al, 2022). Cell lines are commonly used for hypothesis-driven research, such as testing gene knockouts, overexpression studies, or examining the effects of environmental or pharmacological treatments on gene expression. As we demonstrated, the gene expression profiles of cell lines can differ significantly from those observed in native tissues due to alterations in cell signaling, metabolism, and other factors that occur during long-term culture. Thus, findings from cell lines may not always translate to in vivo contexts. The single-cell RNAseq seems more precise than conventional bulk RNAseq

results, since the transcriptome of specific cell groups in a mixture of cells (tissue) can be revealed (Birnbaum, 2018).

Conclusions

In this study, we investigated whether myostatin gene expression inhibition is regulated similarly in mouse skeletal muscle tissue and a myogenic cell line. Even though the results revealed numerous differentially expressed genes and significant pathways at both tissue and cell levels, due to the heterogeneous nature of tissues, which are usually a mixture of different cells, only a few common genes were differentially expressed. It can be recommended that to compare a tissue with a cell line at the transcriptome level, the single-cell RNA sequencing method may have greater accuracy in results.

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Conflict of Interests
None.

References

Ahmad F., Seidman J. and Seidman C. E. (2005) The genetic basis for cardiac remodeling. Annual review of genomics and human genetics 6: 185-216.

Birnbaum K. D. (2018) Power in numbers: single-cell RNA-seq strategies to dissect complex tissues. Annual review of genetics 52, 203-221.

Bogdanovich S., Krag T. O., Barton E. R., Morris L. D., Whittemore L.-A., Ahima R. S., et al. (2002) Functional improvement of dystrophic muscle by myostatin blockade. Journal of Nature 420(6914): 418-421.

Bowen T. S., Schuler G. and Adams V. (2015) Skeletal muscle wasting in cachexia and sarcopenia: molecular pathophysiology and impact of exercise training. Journal of cachexia, sarcopenia and muscle 6(3): 197-207.

Cassar-Malek I., Passelaigue F., Bernard C., Léger J. and Hocquette J.-F. (2007) Target genes of myostatin loss-of-function in muscles of late bovine fetuses. Journal of BMC Genomics 8(1): 1-11.

Ekblom R. and Galindo J. (2011) Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity 107(1): 1-15.

Freire P. P., Fernandez G. J., Cury S. S., De Moraes D., Oliveira J. S., De Oliveira G., et al. (2019) The pathway to cancer cachexia: microRNA-regulated networks in muscle wasting based on integrative meta-analysis. International Journal of Molecular Sciences 20(8):1962.

Gorji A. E., Roudbari Z., Sadeghi B., Javadmanesh A. and Sadkowski T. (2019) Transcriptomic analysis on the promoter regions discover gene networks involving mastitis in cattle. Microbial Pathogenesis 137: 103801.

Güller I. and Russell A. P. (2010) MicroRNAs in skeletal muscle: their role and regulation in development, disease and function. The Journal of Physiology 588(21): 4075-4087.

Hentzen E. R., Lahey M., Peters D., Mathew L., Barash I. A., Fridén J., et al. (2006) Stress-dependent and-independent expression of the myogenic regulatory factors and the MARP genes after eccentric contractions in rats. The Journal of Physiology 570(1): 157-167.

Huang P., Pang D., Wang K., Xu A., Yao C., Li M., et al. (2019) The possible role of complete loss of myostatin in limiting excessive proliferation of muscle cells (C2C12) via activation of microRNAs. International Journal of Molecular Sciences 20(3):643.

Javadmanesh A. and Riasi M. (2023) Analysis of genes related to myostatin and muscle growth using mRNA expression data of C2C12 muscle cell line. In The 10th National and 2nd International Congress on animal science congress of Iran.

Kambadur R., Sharma M., SmithT. P. and Bass J. J. (1997) Mutations in myostatin (GDF8) in double-muscled Belgian Blue and Piedmontese cattle. Journal of Genome Research 7(9): 910-915.

Kemp T., Sadusky T., Saltisi F., Carey N., Moss J., Yang S., et al. (2000) Identification of Ankrd2, a novel skeletal muscle gene coding for a stretch-responsive ankyrin-repeat protein. Genomics 66(3): 229-241.

Kojic S., Medeot E., Guccione E., Krmac H., Zara I., Martinelli V., et al. (2004) The Ankrd2 protein, a link between the sarcomere and the nucleus in skeletal muscle. Journal of Molecular Biology 339(2):313-325.

Lafzi A., Moutinho C., Picelli S. and Heyn H. (2018) Tutorial: guidelines for the experimental design of single-cell RNA sequencing studies. Nature Protocols 13(12): 2742-2757.

Maeda Y. (2019) P. 135Chemokine CXCL12 as an intrinsic modulator of skeletal muscle mass in healthy humans. Neuromuscular Disorders 29: S87.

Martinelli G., Olivari D., Re Cecconi A., Talamini L., Ottoboni L., Lecker S., et al. (2016) Activation of the SDF1/CXCR4 pathway retards muscle atrophy during cancer cachexia. Oncogene 35(48): 6212-6222.

McKoy G., Hou Y., Yang S. Y., Vega Avelaira D., Degens H., Goldspink G. and Coulton, G. R. (2005) Expression of Ankrd2 in fast and slow muscles and its response to stretch are consistent with a role in slow muscle function. Journal of Applied Physiology 98(6): 2337-2343.

Miao Y., Yang J., Xu Z., Jing L., Zhao S. and Li X. (2015) RNA sequencing identifies upregulated kyphoscoliosis peptidase and phosphatidic acid signaling pathways in muscle hypertrophy generated by transgenic expression of myostatin propeptide. International Journal of Molecular Sciences 16(4): 7976-7994.

Miryala S. K., Anbarasu A. and Ramaiah S. (2018) Discerning molecular interactions: a comprehensive review on biomolecular interaction databases and network analysis tools. Gene 642: 84-94.

Mutryn M. F., Brannick E. M., Fu W., Lee W. R. and Abasht B. (2015) Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. BMC Genomics 16(1): 1-19.

Riasi M., Mozaffari Jovin S. and Javadmanesh A. (2024) The effect of modification of DNA interference on myostatin gene expression in mice. Journal of Genetics 103: 02.

Riasi M., Mozaffari Jovin S. and Javadmanesh A. (2022) Effect of Intramuscular and Intraperitoneal Injections of conjugated MSTN-siRNA-cholesterol on Inhibition of Myostatin Gene expression. J Journal of Cell Molecular Research 14(1): 20-27.

Saedi N., Aminafshar M., Chamani M., Honarvar M. and Javadmanesh A. (2022) Expression pattern and network visualization of genes involved in milk persistency in bovine mammary tissue. Agriculture Natural Resources 56(1): 23–34.

Saliani M., Jalal R. and Javadmanesh, A. (2022) Differential expression analysis of genes and long non-coding RNAs associated with KRAS mutation in colorectal cancer cells. Scientific reports 12(1), 7965.

Sicinski P., Geng Y., Ryder-Cook A. S., Barnard E. A., Darlison M. G. and Barnard P. J. (1989) The molecular basis of muscular dystrophy in the mdx mouse: a point mutation. Science 244(4912): 1578-1580.

Soleimani S., Sekhavati M. H. and Javadmanesh A. (2019) Sequencing and Bioinformatic Investigation of Introducing a Repressive Micro-RNA Target Sites in the 3'UTR of Myostatin Gene in some Indigenous Sheep Breeds of Iran. J Iranian Journal of Animal Science Research 11(1): 111-119.

Tsukamoto Y., Senda T., Nakano T., Nakada C., Hida T., Ishiguro N., et al. (2002) Arpp, a new homolog of carp, is preferentially expressed in type 1 skeletal muscle fibers and is markedly induced by denervation. Laboratory Investigation 82(5): 645-655.

Wagner K. R., McPherron A. C., Winik N. and Lee S. J. (2002) Loss of myostatin attenuates severity of muscular dystrophy in mdx mice. J Annals of Neurology: Official Journal of the American Neurological Association the Child Neurology Society 52(6): 832-836.

Welle S., Cardillo A., Zanche M. and Tawil R. (2009) Skeletal muscle gene expression after myostatin knockout in mature mice. Physiological Genomics 38(3): 342-350.

Whittemore L.-A., Song K., Li X., Aghajanian J., Davies M., Girgenrath S., et al. (2003) Inhibition of myostatin in adult mice increases skeletal muscle mass and strength. Biochemical Biophysical Research Communications 300(4): 965-971.

Zhao B., Li E. J., Wall R. J. and Yang J. (2009) Coordinated patterns of gene expressions for adult muscle build-up in transgenic mice expressing myostatin propeptide. BMC Genomics 10(1): 1-16.

Supplementary Figures

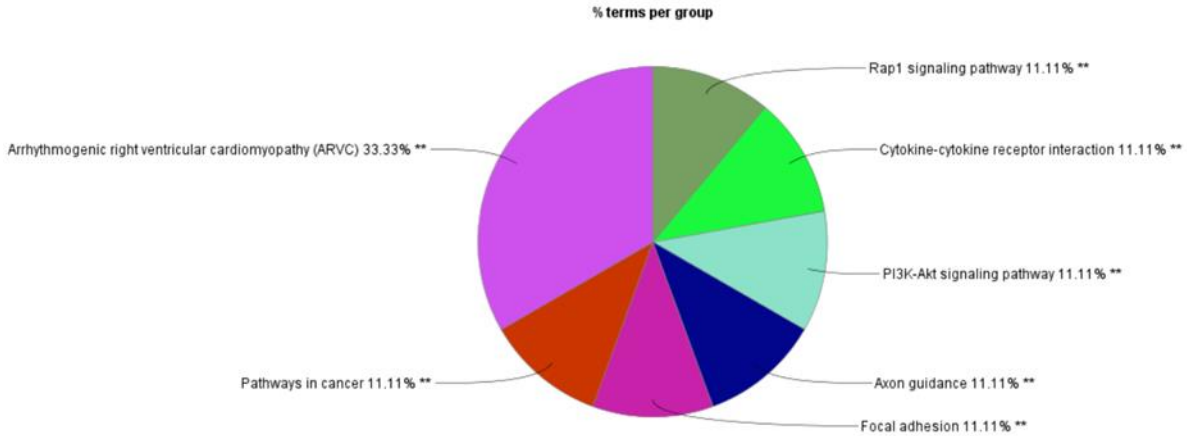

Figure S1. Significant pathways of differentially expressed genes (DEGs) C2C12 cell line

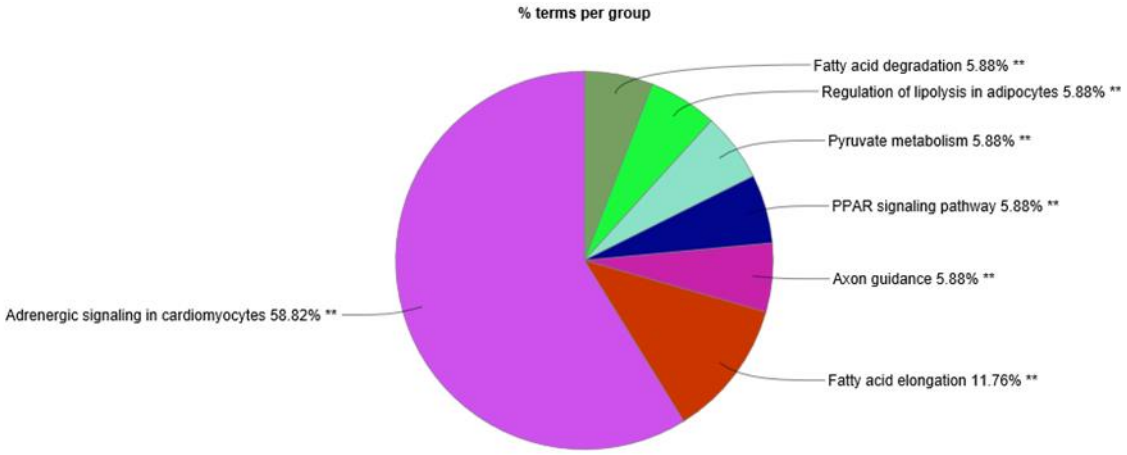

Figure S2. Significant pathways of differentially expressed genes (DEGs) in mouse skeletal muscle tissue.