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Research paper

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Transcriptomic changes in bovine skeletal muscle cells after resveratrol treatment

Dan Hao^{1, 2, 3#}, Xiaogang Wang^{1#}, Xiao Wang², Bo Thomsen³, Haja N. Kadarmideen²,
Xianyong Lan¹, Yongzhen Huang¹, Hong Chen^{1*}

¹ *College of Animal Science and Technology, Northwest A&F University, Shaanxi Key Laboratory of Animal Genetics, Breeding and Reproduction, 712100, Yangling, Shaanxi, China*

² *Quantitative Genomics, Bioinformatics and Computational Biology Group, Department of Applied Mathematics and Computer Science, Technical University of Denmark, 2800, Kongens Lyngby, Denmark*

³ *Department of Molecular Biology and Genetics, Aarhus University, 8000, Aarhus C, Denmark*

These authors contributed equally to this work.

*Correspondence: Hong Chen, Shaanxi Key Laboratory of Animal Genetics, Breeding and Reproduction, College of Animal Science and Technology, Northwest A&F University, Shaanxi 712100, China. E-mail: chenhong1212@263.net.

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Introduction

Skeletal muscles constitute a high proportion of the cellular mass that is essential for the growth traits in livestock species like pig and cattle (Cheek et al., 1971). The growth of skeletal muscle occurs in response to mechanical overload or stimulation by anabolic hormones (Schiaffino et al., 2013).

Resveratrol (RSV) is a natural polyphenol compound that has divergent effects on cell proliferation, differentiation and apoptosis dependent upon several parameters including cell type, organism, RSV dose and oxidative stress (Bosutti and Degens, 2015). Main activities of RSV include anti-oxidant and anti-inflammatory effects as well as the modulation of gene expression and diverse biological processes, for example, altering the functions of histone deacetylase SIRT1 and estrogen receptor (Li et al., 2019; Nwachukwu et al., 2014).

The impact of RSV on muscle mass and strength is complex. RSV is involved in pleiotropic biological activities of muscle such as increasing exercise endurance, enhancing muscle mitochondrial function, improving muscle fatigue resistance, promoting muscle fiber type conversion and protecting against muscle injury (Alway et al., 2017; Joseph et al., 2013; Montesano et al., 2013; Wu et al., 2013; Xiao, 2015). RSV exerts a dose-dependent effect on C2C12 myoblasts, i.e., RSV stimulates cell migration, cell cycle arrest and sprout formation at low doses but inhibits them at higher doses (Bosutti and Degens, 2015). In addition, RSV promotes muscle differentiation and hypertrophy by controlling muscle-specific proteins like myogenic regulatory factors (MRFs) (Montesano et al., 2013). However, RSV does not attenuate the decreases in muscle mass after the disuse by hindlimb suspension (Bennett et al., 2013). Furthermore, RSV supplementation combined with muscle resistance exercise shows synergistic increase of performance and endurance (Alway et al., 2017; Kan et al., 2018), and improves the muscle mass in response to the reloading after hindlimb suspension (Bennett et al., 2013).

Until now, many studies have been conducted to reveal differentially expressed genes (DEGs) in beef muscle associated with diverse traits using RNA sequencing (RNAseq) data (Berton et al., 2016; Gonçalves et al., 2018; Khansefid et al., 2017; Liu et al., 2015; Wærp et al., 2018). So far, to the best of our knowledge very few, if any (e.g., in rats (Zhou et al., 2019), in pigs (Vitali et al., 2019)), studies have analyzed bovine muscle transcriptomes in response to treatment with RSV. Therefore, the aim of this study was to conduct a genome-wide transcriptome study on beef skeletal muscle cells in the absence and presence of RSV to reveal differentially expressed genes. The data underscores the pleiotropic impact of RSV on muscle biology.

Materials and methods

Beef muscle cell culture

Primary myoblasts were isolated from fetal beef *longissimus dorsi* muscle following Miyake's method (Miyake et al., 2012). Then, the isolated cells were cultivated in the high-glucose Dulbecco's modified Eagle's medium (DMEM; Gibco, US) containing 1% penicillin-streptomycin and 20% fetal beef serum (growth medium [GM]; TransGen, Beijing, China). Subsequently, we induced the differentiation of primary bovine myoblast with DMEM containing 2% horse serum (differentiation medium [DM]; HyClone, US) after cells reaching nearly 90% confluence.

Kaminski et al. (2012) found that a certain concentration of RSV dosage (i.e., 30 μM) resulted in a slight decrease of viable C2C12 cell number, while this decreasing effect became more pronounced at the dosage of 75 μM . However, they found that 20 μM RSV did not induce any significant toxicity after the evaluation using lactate dehydrogenase (LDH) assay. In addition, less than 2% of apoptotic cells was found at 20 μM RSV, as they did not accompany by an induction of an apoptotic process (Kaminski et al., 2012). Therefore, our study chose 20 μM dosage of RSV. Following the RSV formulating method in the previous study (Li et al., 2015), 10^5 μM mother solution was formulated by using 22.8 mg RSV (Molar mass = 228) (Sangon Biotech, Shanghai, China) dissolved into 1 mL Dimethyl sulfoxide (DMSO; Sangon Biotech, Shanghai, China), so DMSO is the solution here. Afterwards, 1 μL mother solution and 1 μL pure DMSO were then attenuated into 5 mL DM. One group of cells were treated with constant doses of 20 μM RSV in DM (i.e., 1 μL mother solution added in 5 mL DM), which is the case group. Meanwhile, another group of cells were treated with 1 μL DMSO as the control group.

The previous studies revealed that the primary myoblasts would develop to myofiber through proliferation stage (i.e., 0h culture), determination stage (i.e., 0-24h culture), differentiation stage (i.e., 24-72h culture) and maturation stage (i.e., 72-96h) (Bentzinger et al., 2012; Montesano et al., 2013; Perry and Rudnick, 2000). Therefore, our study constantly treated the

cells of two groups (i.e., case and control groups) for four-day cell differentiations and maturations. The cells were harvested using TRIzol reagent (Takara, Japan) at the point in time of 96h. Three independent experiments of muscle cells (i.e., primary myoblasts) cultures were performed for both case and control treatment groups, separately, so each treatment group had three replicates.

Transcriptomic mRNA sequencing, quality control and alignment

A total amount of 1 µg RNA per sample was used as input material for RNA preparation. RNA sequencing libraries were generated using NEBNext (NEB, USA) for Illumina following manufacturer's recommendations. Messenger RNA (mRNA) was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in NEBNext. After adenylation of 3' ends of DNA fragments, NEBNext adaptors with hairpin loop structure were ligated to prepare for hybridization. In order to select cDNA fragments of 250 ~ 300 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). Hereafter, they were sequenced on an Illumina Hiseq platform to generate the RNAseq of 125 bp or 150 bp paired-end reads.

Clean reads were obtained by removing raw reads with adapter, ploy-N (Percentage of uncertain N bigger than 10%) and low quality (Percentage of $Q_{\text{phred}} \leq 20$ bigger than 50%). The adapters of RNA 5' and RNA 3' used in the sequencing of this study were 5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGA TCT-3' and 5'-GATCGGAAGAGCACACGTCTGAACTCCAGTCAC(barcode)ATCTCGTATGCCGTCT TCTGCTTG-3', respectively. Paired-end clean reads were aligned to the reference genome of *Bos taurus* (UMD_3.1.1/bosTau8) using Hisat2 software (version 2.0.4) (Kim et al., 2015).

Differentially expressed gene analysis

HTSeq software (version 0.9.1) (Anders et al., 2015) was used to count the read numbers. Then, expected number of fragments per kilobase of transcript sequence per millions base pairs sequenced (FPKM) of each gene was calculated based on the length of the gene and read counts

mapped to this gene. Currently, FPKM is the most commonly used method for estimating gene expression levels, because it considers the effect of sequencing depth and gene length for the read count at the same time (Trapnell et al., 2010). The averaged FPKM values for three replicates in two groups were calculated and we set FPKM value > 1 as the threshold of gene expression in this study. DEG analysis of two groups was performed using the R package DESeq (version 1.18.0) (Anders and Huber, 2010) after the normalization of read count. DESeq provides statistical routines for determining differential gene expression in RNAseq data using a model based on the negative binomial distribution that is $K_{ij} \sim \text{NB}(\mu_{ij}, \sigma_{ij}^2)$, where K_{ij} is the read count of gene i of sample j . The resulting P -values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate (FDR). Genes with an adjusted P -value < 0.05 were assigned as DEGs. In addition, fold change (FC) of each gene was calculated based on the averaged FPKM value for three replicates of case group divided by the averaged FPKM value for three replicates of control group, thus, the up-regulated and down-regulated genes were defined when the $\log_2(\text{FC})$ values were positive and negative, respectively.

Differentially expressed gene validation by qRT-PCR experiment

A quantitative reverse transcription polymerase chain reaction (qRT-PCR) approach was applied to validate 19 selected DEGs using the cell samples from the same culture medium as for RNAseq (i.e., one cell culture medium was divided into two parts: one part was used for RNAseq and the other part was used for qRT-PCR). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was chosen as the internal gene for qRT-PCR and SYBR Green Master Mix Reagen kit (GenStar, Beijing) was used to perform qRT-PCR. The first strand of cDNA was erased by StarScript II One-step RT-PCR Kit (Takara, Japan) with 2 μg RNA as the template. The method of $2^{-\Delta\Delta C_t}$ was used to calculate the relative gene expression level. All the primers for qRT-PCR are shown in the supplementary table 2.

Gene Ontology and pathway enrichment

To investigate the associations between the identified DEGs and gene-related biological functions, this study conducted both Gene Ontology (GO) terms and pathways analysis to investigate the important enrichments. R package clusterProfiler (version 3.6) (Yu et al., 2012)

was used to test the statistical enrichment of DEGs in GO terms and KEGG pathways. We used DEGs (both adjusted P -value < 0.05 and adjusted P -value < 0.0001) to enrich the GO terms and pathways.

Protein-protein interactive analysis of DEGs

Based on the STRING database (<https://string-db.org/>), protein-protein interactions (PPIs) were predicted for the top 600 DEGs in this study. Blastx software (version 2.2.28) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to align the target gene sequences to the selected reference protein sequences using the default setting. Afterwards, the networks were built according to the known interactions of *Bos taurus* species. Finally, we used Cytoscape software (version 3.5.1) to visualize the networks of PPIs with the string score above 700, so the number of DEGs involved in the visualization of networks would be less than 600. In the visualized networks, node size and node color were used to indicate the degree and betweenness centrality, respectively.

Results

Briefly, primary myoblasts isolated from fetal beef *longissimus dorsi* muscle were cultivated in DMEM and subsequently induced to differentiate in DM until the degree of cell fusion reaching approximately 90% confluence. Three independent experiments were performed with cell cultures in triplicate. Differentiating myoblasts were grown in the absence or presence of RSV. RNA was isolated and subjected to RNA sequencing. The raw data was quality controlled by removing reads containing adapter, reads containing ploy-N and low-quality reads. Clean reads were mapped to the reference genome of *Bos taurus* (UMD_3.1.1/bosTau8). Differentially expressed gene (DEG) analysis was performed using R package DESeq (Anders and Huber, 2010) and P -values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate (FDR). DEGs were determined using an adjusted P -value < 0.05 of FDR. Gene Ontology (GO) and pathway enrichment analysis were conducted to achieve significant utilities of biological system (adjusted P -value < 0.05). Protein-protein

interactions (PPIs) were predicted in order to reveal networks among the differentially expressed proteins.

Statistics of alignment with bovine reference genome

After four days' incubation, the RNA was purified from the cells and subjected to next-generation sequencing. On average, 59,344,233 clean reads were retained after quality control and 53,759,430 of them were uniquely mapped to the bovine reference genome. Additionally, 45.17 % and 45.42 % of the uniquely aligned reads were mapped to '+' and '-' strands, respectively (Table 1). The results of FPKM value distribution showed that the largest percentages of fragment interval were 0 ~ 1, followed by the fragment interval of 3 ~ 5 and fragment interval of 15 ~ 60 (Supplementary table 1). The Pearson correlation coefficients (PCCs) of gene expression levels among six samples were all above 0.95, which indicated that the expression patterns of the replicates were highly similar (Supplementary Figure 1).

Differentially expressed genes

Based on a threshold of FPKM value > 1 , the number of expressed genes that were common for RSV and DMSO groups was 12,879, which was much higher than the expressed gene number in each separate group (Figure 1A). After statistical test, a total of 3,856 DEGs were identified, comprising 1,805 up-regulated DEGs and 2,051 down-regulated DEGs (adjusted P -value < 0.05) (Figure 1B). Furthermore, using an adjusted P -value < 0.0001 revealed 450 up-regulated DEGs and 681 down-regulated DEGs. The details of up/down-regulated DEGs are listed in Supplementary file 1. We found that the most significant DEGs were the up-regulated gene phospholipid transfer protein (*PLTP*) (adjusted P -value = $9.46\text{E-}174$), followed by the up-regulated gene cyclin-dependent kinase inhibitor (*CDKN1A*) (adjusted P -value = $2.50\text{E-}104$). In the down-regulated category, the most significant gene was 3-hydroxy-3-methylglutaryl-CoA synthase 1 (*HMGCS1*) (adjusted P -value = $1.39\text{E-}95$) (Table 2). Based on FPKM values, we created a heat map to visualize expression levels of DEGs between RSV and DMSO groups, and the DEGs were divided into various classification in the bar (Figure 1C). In addition, we validated the top 19 DEGs by qRT-PCR experiments and the results showed that the expression of *PLTP* was almost 98 times higher in RSV group than in the DMSO group. The other 8 up-

regulated genes were at least 3.5 times higher, while the 10 down-regulated genes were at least 0.1 times lower in RSV group when compared with DMSO group (Figure 2A and 2B).

Significant GO terms

DEGs were subjected to GO enrichment analysis for biological interpretation. Some genes were unmapped in the database leaving 2656 DEGs for analysis that includes 1242 up-regulated and 1414 down-regulated DEGs (adjusted P value < 0.05). Among the 2656 DEGs, we revealed 72 significant GO terms using a cut off at adjusted P -value < 0.05 (Supplementary file 2). Table 3 shows the top ten most significant GO terms of the biological processes category, which underscores the impact RSV on actin cytoskeletal structures in muscle cells and pathways associated with generation of nucleoside phosphates. In the biological process category (Supplementary file 2), 134 genes were assigned to anatomical structure morphogenesis (GO:0009653), 140 genes to phosphorylation (GO:0016310) and 100 genes to tissue development (GO:0009888). Additionally, 115 genes were assigned to cytoskeletal part (GO:0044430) in the cellular component category, whereas 133 genes were associated with drug binding (GO:0008144) in the molecular function category. Interestingly, the up-regulated genes were only enriched in two GO terms (i.e., DNA-dependent DNA replication (GO:0006261) and DNA replication (GO:0006260)) of the biological processes category (Figure 3 and Supplementary file 2). The down-regulated genes were enriched in the GO terms of actin cytoskeleton organization (GO:0030036) and actin filament-based process (GO:0030029) (Figure 3 and Supplementary file 2). If considering 1131 DEGs filtered by an adjusted P -value < 0.0001 , we found 45 significant GO terms (adjusted P -value < 0.01) including 33 biological process, 7 cellular component and 5 molecular function with the details in Supplementary file 2.

Significant KEGG pathways

The same DEGs as used in GO term analysis were selected for KEGG pathways analysis, which showed significantly enrichment in pathways like MAPK signaling pathway (bta04010), rap1 signaling pathway (bta04015) and PI3K-AKT signaling pathway (bta04151) (Figure 4 and Table 4). Separation into up- and down- regulated DEGs revealed that the top three significant

enriched pathways of up-regulated genes were DNA replication (bta03030), fanconi anemia pathway (bta03460), p53 signaling pathway (bta04115), while the top three significant enriched pathways of downregulated genes were focal adhesion (bta04510), regulation of actin cytoskeleton (bta04810), proteoglycans in cancer (bta05205) (Figure 4 and Supplementary file 2). If considering 1131 DEGs filtered by an adjusted P -value < 0.0001 , we found 28 significant pathways (adjusted P -value < 0.01), and 45 genes were enriched in PI3K-Akt signaling pathway including 16 upregulated genes and 29 downregulated genes with the details in Supplementary file 2.

Network of PPIs for DEGs

To further understand the transcriptome changes resulting from RSV treatment, we analyzed for interactions among the proteins encoded by DEGs. Our study set the string score of the final visualized networks above 700, so less DEGs were able to show in the visualization. Thus, up-regulated DEGs displayed only one large protein-protein network in which *CDKN1A* was strongly connected with origin recognition complex subunit 1 (*ORC1*), phosphoinositide-3-kinase regulatory subunit 2 (*PIK3R2*) and MET proto-oncogene receptor tyrosine kinase (*MET*) (Figure 5A). The down-regulated DEGs were assembled into six clusters. One of the largest clusters consisted of the highly-connected thrombospondin 3 (*THBS3*), which interacted with many other adhesion and matrix proteins including syndecan 3 (*SDC3*), integrin subunit beta 1 (*ITGB1*), collagen type III alpha 1 chain (*COL3A1*), integrin subunit alpha V (*ITGAV*), collagen type VI alpha 2 chain (*COL6A2*), integrin subunit beta 5 (*ITGB5*), collagen type V alpha 1 chain (*COL5A1*) and *THBS1,2,4*. The remaining five smaller protein clusters focused on eukaryotic translation initiation factor 4E (*EIF4E*), actin related protein 2/3 complex subunit 3 (*ARPC3*), cytochrome P450, family 51, subfamily A, polypeptide 1 (*CYP51A1*), platelet derived growth factor receptor alpha (*PDGFRA*) and coagulation factor XIII A chain (*F13A1*) (Figure 5B). All the selected top 600 DEGs with string scores are listed in Supplementary file 1.

Discussion

Resveratrol is a natural constituent of red grapes and berries due to its pleiotropic bioactivities. RSV has been considered to have general beneficial health effects and therapeutic potential for human diseases (Baur and Sinclair, 2006). For example, RSV has been observed to exert antitumor effects by suppressing angiogenesis, which is important for tumor growth and progression (Kimura and Okuda, 2001; Tseng et al., 2004). In this context, it is noteworthy that we observed significantly down-regulated expression of genes associated with blood vessel and cardiovascular system development resulting from RSV exposure. Likewise, our data show a significant impact of RSV on vascular smooth muscle contraction (bta04270; Supplemental file 2), which functions to regulate blood flow and pressure, thereby having potentially favorable effects on cardiovascular health. Furthermore, RSV has been shown to affect aging and extend lifespan of diverse organisms, including yeast (*S. cerevisiae*), worm (*C. elegans*), fly (*D. melanogaster*) and fish (*N. furzeri*) by mimicking caloric restriction primarily via regulation of a family of NAD⁺-dependent deacetylases, although additional genes may play a role (Baur et al., 2006; Hector et al., 2012; Lee et al., 2019). In agreement with the results, we found that RSV up-regulated *SIRT1* and *SIRT3* expression and down-regulated *SIRT2* in muscle cells (Supplemental file 1). The previous studies found that epigallocatechin-3-gallate (EGCG) accounts for 45–65% of the catechinic acid content as the most effective component of tea polyphenols (Tanaka et al., 2005). EGCG is a powerful anti-oxidant and plays key roles in anti-cancers and anti-vascular diseases. In addition, it can also inhibit the proliferation of aortic smooth muscle cells in human (Afzal et al., 2015; Shu et al., 2014). From the results of our study (Supplemental file 2) and other studies (Kimura and Okuda, 2001; Li et al., 2019; Nwachukwu et al., 2014; Tseng et al., 2004), resveratrol demonstrated the similar biological activities, so polyphenol compounds is suggested to change the expressions of genes involved in the similar regulatory molecular mechanisms of muscle cells. However, further study is reasonably necessary to identify the expression levels of specific genes if we use catechinic acid in the treatment group.

In our transcriptome data, *PLTP* was 28-fold up-regulated (adjusted *P*-value = 9,46E-174) by RSV treatment, which was validated by qRT-PCR results. Previous studies have shown that *PLTP* was expressed in a wide variety of tissues (e.g., muscle) to mediate a net transfer

of phospholipids between vesicles and plasma (Jauhiainen and Ehnholm, 2005). Additionally, *PLTP* played a role in cholesterol and fatty acid metabolism (Beyer et al., 2004), and was involved in the variations of muscle lipid composition and flavor in beef (Dunner et al., 2013; Qin et al., 2015). Another highly significant gene, *CDKN1A*, is known to play an important part in cell cycle regulation. Notably, the *CDKN1A* mRNA is post-transcriptionally regulated by interaction with miR-208b to control cell cycle and proliferation of cattle primary skeletal muscle cell (Wang et al., 2019). In our results, *CDKN1A* was enriched in eight KEGG pathways including the PI3K-AKT signaling pathway (bta04115), which plays an important role during myoblast differentiations (Elia et al., 2007). In addition, *CDKN1A* has a central function in the protein network encoded by genes that were up-regulated by RSV (Figure 5A). Among the interacting partners, *PIK3R2* is a negative regulator of the PI3K-AKT signaling pathway (Luo and Cantley, 2005). A number of reports have previously implicated RSV in modulating the PIK-AKT pathway in various biological systems such as neuroprotection (Zamin et al., 2006) and apoptosis (Fan et al., 2018). Finally, the up-regulated DEGs were enriched in two GO term biological processes related to DNA replication (GO:0006261 and GO:0006260) as well as the KEGG pathway DNA replication (bta03030), suggesting that muscle cell differentiation is modulated by RSV by affecting the cell cycle and myoblast proliferation (Joe et al., 2002; Poussier et al., 2005).

The transcriptome results show that RSV targets retinoic acid signaling in the muscle cells (Supplemental file 1). Thus, we observed the up-regulation of *RAI2* (retinoic acid induced 2) and *RARB* (retinoic acid receptor beta) as well as the down-regulation of *RBPI* (retinol binding protein 1), *RBP4* (retinol binding protein 4), *RAI14* (retinoic acid induced 14), *RARA* (retinoic acid receptor alpha), *RARG* (retinoic acid receptor gamma), *RARRES2* (retinoic acid receptor responder 2), *RDH10* (retinol dehydrogenase 10), *RDH11* (retinol dehydrogenase 11), *RAI1* (retinoic acid induced 1), *CRABP1* (cellular retinoic acid-binding protein 1) and *CRABP2* (cellular retinoic acid-binding protein 2). Retinoic acid has previously been shown to induce growth arrest of myogenic cells (Froeschlé et al., 1996) and promote muscle cell differentiation (Halevy and Lerman, 1993).

The transcriptome changes caused by RSV exposure are highly enriched in GO terms and KEGG pathways associated with actin cytoskeleton organization, focal adhesion and extracellular matrix-receptor interactions. Notably, one significant GO term, tissue development (GO:0009888), identified in this study (Supplementary file 2) was associated with myogenic and osteogenic differentiation of adipose tissue-derived stem cells in human (Quan et al., 2016). The tissue development GO term was also found in the enrichment analysis for the gilthead seabream when compared to all seabream 454 sequences to the novel EST sequences (Yúfera et al., 2012) and fibroblasts in the scarred rat (Kishimoto et al., 2016). In addition, the drug binding (GO:0008144) in this study (Supplementary file 2) is the significant GO term in *schistosomula mansonii* after c-Jun N-terminal kinase knockdown where the down-regulated DEGs were enriched (Gava et al., 2019). Also, PI3K-AKT signaling pathway (bta04151) (Supplementary file 2) plays a key role in the dynamic switch between distinct KSRP functions which is favorable for skeletal myogenesis (Briata et al., 2012), while reducing the activities of this pathway in skeletal muscle could result in intrauterine growth restriction (Xing et al., 2019). The protein-protein analysis showed large networks between proteins engaged in cell-cell and cell-matrix interactions such as thrombospondin 1-4, syndecans-2 and -3, collagens, as well as alpha and beta integrin subunits. Collectively, these observations underscore that RSV has a strong impact on diverse cellular components and processes, including proliferation, differentiation and migration of muscle cells, contacts between cells and the extracellular matrix, maintenance and function of the actin cytoskeleton. Furthermore, we found evidence of RSV effects on several signaling pathways. Prominent examples are Wnt signaling pathway (cell-fate specification), Hippo signaling pathway (organ size control), MAPK signaling pathway (cell proliferation, differentiation, motility), p53 signaling pathway (cell cycle control, apoptosis), Rap1 signaling pathway (cell adhesion, cell-cell junction formation) and PI3k-Akt signaling pathway (diverse cellular functions).

In conclusion, this study provided an improved understanding of the molecular mechanisms underlying the impact of resveratrol on muscle cells at the transcriptomic level, and revealed a list of differentially expressed genes, significant GO terms and KEGG pathways responding to resveratrol treatment.

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Contributions

DH performed all the experiments with the help of XGW. XGW performed qRT-PCR experiments. DH and XW analyzed the data and drafted the manuscript. BT, XW and HNK improved the manuscript. XYL and YZH helped to collect the bovine samples for the experiment. HC conceived and designed the experiments. #DH and #XGW contributed equally to this manuscript. All authors read and approved the final manuscript.

References

- Afzal, M., Safer, A.M., Menon, M., 2015. Green tea polyphenols and their potential role in health and disease. *Inflammopharmacology*. <https://doi.org/10.1007/s10787-015-0236-1>
- Alway, S.E., McCrory, J.L., Kearcher, K., Vickers, A., Frear, B., Gilleland, D.L., Bonner, D.E., Thomas, J.M., Donley, D.A., Lively, M.W., Mohamed, J.S., 2017. Resveratrol Enhances Exercise-Induced Cellular and Functional Adaptations of Skeletal Muscle in Older Men and Women. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences* 72, 1595–1606. <https://doi.org/10.1093/gerona/glx089>
- Anders, S., Huber, W., 2010. Differential expression analysis for sequence count data. *Genome Biology* 11. <https://doi.org/10.1186/gb-2010-11-10-r106>
- Anders, S., Pyl, P.T., Huber, W., 2015. HTSeq-A Python framework to work with high-throughput sequencing data. *Bioinformatics* 31, 166–169. <https://doi.org/10.1093/bioinformatics/btu638>
- Baur, J.A., Pearson, K.J., Price, N.L., Jamieson, H.A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J.S., Lopez-Lluch, G., Lewis, K., Pistell, P.J., Poosala, S., Becker, K.G., Boss, O., Gwinn, D., Wang, M., Ramaswamy, S., Fishbein, K.W., Spencer, R.G., Lakatta, E.G., Le Couteur, D., Shaw, R.J., Navas, P., Puigserver, P., Ingram, D.K., De Cabo, R., Sinclair, D.A., 2006. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 444, 337–342. <https://doi.org/10.1038/nature05354>

- Baur, J.A., Sinclair, D.A., 2006. Therapeutic potential of resveratrol : the in vivo evidence. *Nature Reviews Drug Discovery* 5, 493–506. <https://doi.org/10.1038/nrd2060>
- Bennett, B.T., Mohamed, J.S., Alway, S.E., 2013. Effects of Resveratrol on the Recovery of Muscle Mass Following Disuse in the Plantaris Muscle of Aged Rats. *PLoS ONE* 8, 1–19. <https://doi.org/10.1371/journal.pone.0083518>
- Bentzinger, C.F., Wang, Y.X., Rudnicki, M.A., 2012. Building muscle: molecular regulation of myogenesis. *Cold Spring Harbor perspectives in biology*. <https://doi.org/10.1101/cshperspect.a008342>
- Berton, M.P., Fonseca, L.F.S., Gimenez, D.F.J., Utembergue, B.L., Cesar, A.S.M., Coutinho, L.L., de Lemos, M.V.A., Aboujaoude, C., Pereira, A.S.C., Silva, R.M. d. O., Stafuzza, N.B., Feitosa, F.L.B., Chiaia, H.L.J., Olivieri, B.F., Peripolli, E., Tonussi, R.L., Gordo, D.M., Espigolan, R., Ferrinho, A.M., Mueller, L.F., de Albuquerque, L.G., de Oliveira, H.N., Duckett, S., Baldi, F., 2016. Gene expression profile of intramuscular muscle in Nellore cattle with extreme values of fatty acid. *BMC Genomics* 17, 1–16. <https://doi.org/10.1186/s12864-016-3232-y>
- Beyer, T.P., Schmidt, R.J., Foxworthy, P., Zhang, Y., Dai, J., Bensch, W.R., Kauffman, R.F., Gao, H., Ryan, T.P., Jiang, X.C., Karathanasis, S.K., Eacho, P.I., Cao, G., 2004. Coadministration of a liver X receptor agonist and a peroxisome proliferator activator receptor- α agonist in mice: Effects of nuclear receptor interplay on high-density lipoprotein and triglyceride metabolism in vivo. *Journal of Pharmacology and Experimental Therapeutics* 55, 1172–1178. <https://doi.org/10.1124/jpet.103.064535>
- Bosutti, A., Degens, H., 2015. The impact of resveratrol and hydrogen peroxide on muscle cell plasticity shows a dose-dependent interaction. *Scientific Reports* 5. <https://doi.org/10.1038/srep12192>
- Briata, P., Lin, W.J., Giovarelli, M., Pasero, M., Chou, C.F., Trabucchi, M., Rosenfeld, M.G., Chen, C.Y., Gherzi, R., 2012. PI3K/AKT signaling determines a dynamic switch between distinct KSRP functions favoring skeletal myogenesis. *Cell Death and Differentiation*. <https://doi.org/10.1038/cdd.2011.117>
- Cheek, D.B., Holt, A.B., Hill, D.E., Talbert, J.L., 1971. Skeletal muscle cell mass and growth: The concept of the deoxyribonucleic acid unit. *Pediatric Research* 5, 312–328. <https://doi.org/10.1203/00006450-197107000-00004>
- Dunner, S., Sevane, N., Garcia, D., Leveziel, H., Williams, J.L., Mangin, B., Valentini, A., Consortium, G., 2013. Genes involved in muscle lipid composition in 15 European *Bos taurus* breeds. *Animal Genetics* 44, 493–501. <https://doi.org/10.1111/age.12044>
- Elia, D., Madhala, D., Ardon, E., Reshef, R., Halevy, O., 2007. Sonic hedgehog promotes proliferation and differentiation of adult muscle cells: Involvement of MAPK/ERK and PI3K/Akt pathways. *Biochimica et Biophysica Acta - Molecular Cell Research* 1773, 1438–1446. <https://doi.org/10.1016/j.bbamcr.2007.06.006>

- Fan, Y., Chiu, J.F., Liu, J., Deng, Y., Xu, C., Zhang, J., Li, G., 2018. Resveratrol induces autophagy-dependent apoptosis in HL-60 cells. *BMC Cancer* 18:581, 1–10.
<https://doi.org/10.1186/s12885-018-4504-5>
- Froeschlé, A., Carnac, G., Alric, S., Montarras, D., Pinset, C., Rochette-Egly, C., Bonniieu, A., 1996. RXR α is essential for mediating the all-trans retinoic acid-induced growth arrest of C2 myogenic cells. *Oncogene* 12, 411–421.
- Gava, S.G., Tavares, N.C., Falcone, F.H., Oliveira, G., Mourão, M.M., 2019. Profiling Transcriptional Regulation and Functional Roles of *Schistosoma mansoni* c-Jun N-Terminal Kinase. *Frontiers in Genetics*. <https://doi.org/10.3389/fgene.2019.01036>
- Gonçalves, T.M., De Almeida Regitano, L.C., Koltes, J.E., Cesar, A.S.M., Da Silva Andrade, S.C., Mourão, G.B., Gasparin, G., Moreira, G.C.M., Fritz-Waters, E., Reecy, J.M., Coutinho, L.L., 2018. Gene Co-expression Analysis Indicates Potential Pathways and Regulators of Beef Tenderness in Nellore Cattle. *Frontiers in Genetics* 9, 1–18.
<https://doi.org/10.3389/fgene.2018.00441>
- Halevy, O., Lerman, O., 1993. Retinoic acid induces adult muscle cell differentiation mediated by the retinoic acid receptor- α . *Journal of Cellular Physiology* 154, 566–572.
<https://doi.org/10.1002/jcp.1041540315>
- Hector, K.L., Lagisz, M., Nakagawa, S., 2012. The effect of resveratrol on longevity across species: a meta-analysis. *Biology Letters* 8, 790–793.
<https://doi.org/10.1098/rsbl.2012.0316>
- Jauhiainen, M., Ehnholm, C., 2005. Determination of human plasma phospholipid transfer protein mass and activity. *Methods* 36, 97–101.
<https://doi.org/10.1016/j.ymeth.2004.11.006>
- Joe, A.K., Liu, H., Suzui, M., Vural, M.E., Xiao, D., Weinstein, I.B., Joe, A.K., Weinstein, I.B., 2002. Resveratrol Induces Growth Inhibition, S-phase Arrest, Apoptosis, and Changes in Biomarker Expression in Several Human Cancer Cell lines. *Clinical Cancer Research* 8, 893–903. <https://doi.org/10.1158/1078-0432.CCR-01-0500> Published March 2002
- Joseph, A.M., Malamo, A.G., Silvestre, J., Wawrzyniak, N., Carey-Love, S., Nguyen, L.M.D., Dutta, D., Xu, J., Leeuwenburgh, C., Adhihetty, P.J., 2013. Short-term caloric restriction, resveratrol, or combined treatment regimens initiated in late-life alter mitochondrial protein expression profiles in a fiber-type specific manner in aged animals. *Experimental Gerontology*. <https://doi.org/10.1016/j.exger.2013.05.061>
- Kaminski, J., Lançon, A., Aires, V., Limagne, E., Tili, E., Michaille, J.J., Latruffe, N., 2012. Resveratrol initiates differentiation of mouse skeletal muscle-derived C2C12 myoblasts. *Biochemical Pharmacology*. <https://doi.org/10.1016/j.bcp.2012.08.023>
- Kan, N.W., Lee, M.C., Tung, Y.T., Chiu, C.C., Huang, C.C., Huang, W.C., 2018. The Synergistic Effects of Resveratrol combined with Resistant Training on Exercise Performance and Physiological Adaptation. *Nutrients* 10, 1360.
<https://doi.org/10.3390/nu10101360>

- Khansefid, M., Millen, C.A., Chen, Y., Pryce, J.E., Chamberlain, A.J., Vander Jagt, C.J., Gondro, C., Goddard, M.E., 2017. Gene expression analysis of blood, liver, and muscle in cattle divergently selected for high and low residual feed intake. *Journal of Animal Science* 95, 4764–4775. <https://doi.org/10.2527/jas2016.1320>
- Kim, D., Langmead, B., Salzberg, S.L., 2015. HISAT: a fast spliced aligner with low memory requirements. *Nature Methods* 12, 357–360. <https://doi.org/10.1038/nmeth.3317>
- Kimura, Y., Okuda, H., 2001. Resveratrol Isolated from *Polygonum cuspidatum* Root Prevents Tumor Growth and Metastasis to Lung and Tumor-Induced Neovascularization in Lewis Lung Carcinoma-Bearing Mice. *The Journal of Nutrition* 131, 1844–1849. <https://doi.org/10.1093/jn/131.6.1844>
- Kishimoto, Y., Kishimoto, A.O., Ye, S., Kendzierski, C., Welham, N. V., 2016. Modeling fibrosis using fibroblasts isolated from scarred rat vocal folds. *Laboratory Investigation*. <https://doi.org/10.1038/labinvest.2016.43>
- Lee, S.-H., Lee, J.-H., Lee, H.-Y., Min, K.-J., 2019. Sirtuin signaling in cellular senescence and aging. *BMB reports* 52, 24–34. <https://doi.org/10.5483/BMBRep.2019.52.1.290>
- Li, H., Xia, N., Hasselwander, S., Daiber, A., 2019. Resveratrol and vascular function. *International Journal of Molecular Sciences* 20, 1–16. <https://doi.org/10.3390/ijms20092155>
- Li, M., Sun, X., Zhou, Y., Wei, X., Sun, Y., Lan, X., Lei, C., Chen, H., 2015. Nicotinamide and resveratrol regulate bovine adipogenesis through a SIRT1-dependent mechanism. *Journal of Functional Foods*. <https://doi.org/10.1016/j.jff.2015.08.008>
- Liu, G.F., Cheng, H.J., You, W., Song, E.L., Liu, X.M., Wan, F.C., 2015. Transcriptome profiling of muscle by RNA-Seq reveals significant differences in digital gene expression profiling between Angus and Luxi cattle. *Animal Production Science* 55, 1172–1178. <https://doi.org/doi.org/10.1071/AN14096>
- Luo, J., Cantley, L.C., 2005. The Negative Regulation of Phosphoinositide 3-Kinase Signaling by p85 and Its Implication in Cancer. *Cell Cycle*. <https://doi.org/10.4161/cc.4.10.2062>
- Miyake, M., Takahashi, H., Kitagawa, E., 2012. AMPK activation by AICAR inhibits myogenic differentiation and myostatin expression in Cattle. *Cell and Tissue Research* 349, 615–623. <https://doi.org/10.1007/s00441-012-1422-8>
- Montesano, A., Luzi, L., Senesi, P., Mazzocchi, N., Terruzzi, I., 2013. Resveratrol promotes myogenesis and hypertrophy in murine myoblasts. *Journal of Translational Medicine* 11, 310. <https://doi.org/10.1186/1479-5876-11-310>
- Nwachukwu, J.C., Srinivasan, S., Bruno, N.E., Parent, A.A., Hughes, T.S., Pollock, J.A., Gjyshi, O., Cavett, V., Nowak, J., Garcia-Ordenez, R.D., Houtman, R., Griffin, P.R., Kojetin, D.J., Katzenellenbogen, J.A., Conkright, M.D., Nettles, K.W., 2014. Resveratrol modulates the inflammatory response via an estrogen receptor-signal integration network. *eLife* 2014, 1–30. <https://doi.org/10.7554/eLife.02057>

- Perry, R.L., Rudnick, M.A., 2000. Molecular mechanisms regulating myogenic determination and differentiation. *Frontiers in bioscience : a journal and virtual library*.
<https://doi.org/10.2741/perry>
- Poussier, B., Cordova, A.C., Becquemin, J.P., Sumpio, B.E., 2005. Resveratrol inhibits vascular smooth muscle cell proliferation and induces apoptosis. *Journal of Vascular Surgery* 42, 1190-1190.e14. <https://doi.org/10.1016/j.jvs.2005.08.014>
- Qin, W., Liang, C.N., Guo, X., Chu, M., Pei, J., Bao, P.J., Wu, X.Y., Li, T.K., Yan, P., 2015. PPAR α signal pathway gene expression is associated with fatty acid content in yak and cattle longissimus dorsi muscle. *Genetics and Molecular Research* 14, 14469–14478.
<https://doi.org/10.4238/2015.November.18.9>
- Quan, L., Wang, Y., Liang, J., Qiu, T., Wang, H., Zhang, Ye, Zhang, Yu, Hui, Q., Tao, K., 2016. Screening for genes, transcription factors and miRNAs associated with the myogenic and osteogenic differentiation of human adipose tissue-derived stem cells. *International Journal of Molecular Medicine*. <https://doi.org/10.3892/ijmm.2016.2788>
- Schiaffino, S., Dyar, K.A., Ciciliot, S., Blaauw, B., Sandri, M., 2013. Mechanisms regulating skeletal muscle growth and atrophy. *FEBS Journal* 280, 4294–4314.
<https://doi.org/10.1111/febs.12253>
- Shu, Z., Yu, M., Zeng, G., Zhang, X., Wu, L., Tan, X., 2014. Epigallocatechin-3-gallate inhibits proliferation of human aortic smooth muscle cells via up-regulating expression of mitofusin 2. *European Journal of Cell Biology*.
<https://doi.org/10.1016/j.ejcb.2014.04.001>
- Tanaka, T., Matsuo, Y., Kouno, I., 2005. A novel black tea pigment and two new oxidation products of epigallocatechin-3-O-gallate. *Journal of Agricultural and Food Chemistry*.
<https://doi.org/10.1021/jf0512656>
- Trapnell, C., Williams, B.A., Pertea, G., Mortazavi, A., Kwan, G., Van Baren, M.J., Salzberg, S.L., Wold, B.J., Pachter, L., 2010. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nature Biotechnology* 28, 511–515. <https://doi.org/10.1038/nbt.1621>
- Tseng, S.H., Lin, S.M., Chen, J.C., Su, Y.H., Huang, H.Y., Chen, C.K., Lin, P.Y., Chen, Y., 2004. Resveratrol Suppresses the Angiogenesis and Tumor Growth of Gliomas in Rats. *Clinical Cancer Research* 10, 2190–2202. <https://doi.org/10.1158/1078-0432.CCR-03-0105>
- Vitali, M., Sirri, R., Zappaterra, M., Zambonelli, P., Giannini, G., Fiego, D.P. Lo, Davoli, R., 2019. Functional analysis finds differences on the muscle transcriptome of pigs fed an n-3 PUFA-enriched diet with or without antioxidant supplementations. *PLoS ONE*.
<https://doi.org/10.1371/journal.pone.0212449>
- Wærp, H.K.L., Waters, S.M., McCabe, M.S., Cormican, P., Salte, R., 2018. RNA-seq analysis of bovine adipose tissue in heifers fed diets differing in energy and protein content. *PLoS ONE* 13, 1–21. <https://doi.org/10.1371/journal.pone.0201284>

- Wang, J., Song, C., Cao, X., Li, H., Cai, H., Ma, Yilei, Huang, Y., Lan, X., Lei, C., Ma, Yun, Bai, Y., Lin, F., Chen, H., 2019. MiR-208b regulates cell cycle and promotes skeletal muscle cell proliferation by targeting CDKN1A. *Journal of Cellular Physiology* 234, 3720–3729. <https://doi.org/10.1002/jcp.27146>
- Wu, R.E., Huang, W.C., Liao, C.C., Chang, Y.K., Kan, N.W., Huang, C.C., 2013. Resveratrol protects against physical fatigue and improves exercise performance in mice. *Molecules*. <https://doi.org/10.3390/molecules18044689>
- Xiao, N.N., 2015. Effects of resveratrol supplementation on oxidative damage and lipid peroxidation induced by strenuous exercise in rats. *Biomolecules and Therapeutics*. <https://doi.org/10.4062/biomolther.2015.015>
- Xing, Y., Zhang, J., Wei, H., Zhang, H., Guan, Y., Wang, X., Tong, X., 2019. Reduction of the PI3K/Akt related signaling activities in skeletal muscle tissues involves insulin resistance in intrauterine growth restriction rats with catch-up growth. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0216665>
- Yu, G., Wang, L.G., Han, Y., He, Q.Y., 2012. ClusterProfiler: An R package for comparing biological themes among gene clusters. *OMICS A Journal of Integrative Biology* 16, 284–287. <https://doi.org/10.1089/omi.2011.0118>
- Yúfera, M., Halm, S., Beltran, S., Fusté, B., Planas, J. V., Martínez-Rodríguez, G., 2012. Transcriptomic Characterization of the Larval Stage in Gilthead Seabream (*Sparus aurata*) by 454 Pyrosequencing. *Marine Biotechnology*. <https://doi.org/10.1007/s10126-011-9422-3>
- Zamin, L.L., Dillenburg-Pilla, P., Argenta-Comiran, R., Horn, A.P., Simão, F., Nassif, M., Gerhardt, D., Frozza, R.L., Salbego, C., 2006. Protective effect of resveratrol against oxygen-glucose deprivation in organotypic hippocampal slice cultures: Involvement of PI3-K pathway. *Neurobiology of Disease* 24, 170–182. <https://doi.org/10.1016/j.nbd.2006.06.002>
- Zhou, J., Liao, Z., Jia, J., Chen, J.L., Xiao, Q., 2019. The effects of resveratrol feeding and exercise training on the skeletal muscle function and transcriptome of aged rats. *PeerJ*. <https://doi.org/10.7717/peerj.7199>

Tables, figures and supplementary information

Table 1. Statistics of alignment with reference genome of *Bos taurus* (UMD_3.1.1/bosTau8).

Sample	Clean reads	Total mapped rate	Uniquely mapped rate	Reads mapped to '+' rate	Reads mapped to '-' rate
RSV1	61,467,328	57,253,521 (93.14%)	55,671,493 (90.57%)	27,761,390 (45.16%)	27,910,103 (45.41%)

RSV2	58,041,564	53,952,656 (92.96%)	52,368,969 (90.23%)	26,105,230 (44.98%)	26,263,739 (45.25%)
RSV3	61,730,360	57,513,450 (93.17%)	55,853,521 (90.48%)	27,853,602 (45.12%)	27,999,919 (45.36%)
DMSO1	59,953,152	55,943,895 (93.31%)	54,312,034 (90.59%)	27,079,757 (45.17%)	27,232,277 (45.42%)
DMSO2	60,065,380	56,115,853 (93.42%)	54,572,821 (90.86%)	27,220,741 (45.32%)	27,352,080 (45.54%)
DMSO3	54,807,614	51,173,062 (93.37%)	49,777,739 (90.82%)	24,820,587 (45.29%)	24,957,152 (45.54%)
Mean	59,344,233	55,325,406 (93.23%)	53,759,430 (90.59%)	26,806,885 (45.17%)	26,952,545 (45.42%)
SD	2,583,402	2,393,278 (92.64%)	2,314,970 (89.61%)	1,156,731 (44.78%)	1,158,260 (44.83%)

Table 2. Top ten up/down-regulated differentially expressed genes (DEGs).

DEG	Gene description	Chromosome	Up/down regulation	Log ₂ (FC)	Adjusted P-value
<i>PLTP</i>	Phospholipid transfer protein	13	Up	4.7721	9.46E-174
<i>CDKN1A</i>	Cyclin dependent kinase inhibitor 1A	23	Up	1.971	2.50E-104
<i>MGAT5B</i>	Mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetylglucosaminyltransferase, isozyme B	19	Up	3.3593	7.95E-95
<i>SUSD4</i>	Sushi domain containing 4	16	Up	3.152	4.29E-73
<i>EPHA2</i>	EPH receptor A2	2	Up	1.7419	1.48E-68

<i>RHBDL1</i>	Rhomboid like 1	25	Up	1.6884	6.07E-55
<i>FAM83H</i>	Family with sequence similarity 83 member H	14	Up	1.9811	2.27E-48
<i>SYNE2</i>	Spectrin repeat containing nuclear envelope protein 2	10	Up	1.5573	6.16E-44
<i>ABHD8</i>	Abhydrolase domain containing 8	7	Up	1.4152	9.08E-41
<i>FRMD8</i>	FERM domain containing 8	29	Up	1.2518	3.76E-35
<i>HMGCS1</i>	3-hydroxy-3-methylglutaryl-CoA synthase 1	20	Down	-2.3435	1.39E-95
<i>CIQTNF3</i>	C1q and tumor necrosis factor related protein 3	20	Down	-2.1051	7.79E-89
<i>PRSS35</i>	Protease, serine 35	9	Down	-1.9373	1.70E-83
<i>C1R</i>	Complement C1r	5	Down	-2.0882	4.99E-78
<i>ANPEP</i>	Alanyl aminopeptidase, membrane	21	Down	-2.2004	2.59E-75
<i>ACAT2</i>	Acetyl-CoA acetyltransferase 2	9	Down	-1.7617	3.41E-65
<i>CRABP2</i>	Cellular retinoic acid binding protein 2	3	Down	-1.9769	9.17E-65
<i>KCNK12</i>	Potassium two pore domain channel subfamily K member 12	11	Down	-2.3164	5.92E-63
<i>THBS4</i>	Thrombospondin 4	10	Down	-2.4171	4.62E-57

<i>PODN</i>	Podocan	3	Down	-1.5802	3.83E-54
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Table 3. The top 10 significant GO terms of 2656 differentially expressed genes (DEGs) in biological processes category.

GO term	Description	DEG number	<i>P</i> -value	Adjusted value	<i>P</i> -value
GO:0030036	actin cytoskeleton organization	50	1.43E-06	1.90E-03	
GO:0030029	actin filament-based process	53	1.44E-06	1.90E-03	
GO:0007015	actin filament organization	34	8.55E-06	6.36E-03	
GO:0016126	sterol biosynthetic process	11	9.63E-06	6.36E-03	
GO:0003012	muscle system process	34	1.68E-05	8.88E-03	
GO:0009653	anatomical structure morphogenesis	134	2.50E-05	9.78E-03	
GO:0097435	supramolecular fiber organization	52	3.14E-05	9.78E-03	
GO:1901293	nucleoside phosphate biosynthetic process	40	3.36E-05	9.78E-03	
GO:1902653	secondary alcohol biosynthetic process	10	3.51E-05	9.78E-03	
GO:0009165	nucleotide biosynthetic process	39	3.70E-05	9.78E-03	

Table 4. The top 10 significant pathways of 2656 differentially expressed genes (DEGs).

Pathway	Description	DEG number	<i>P</i> -value	Adjusted value	<i>P</i> -value
bta04510	Focal adhesion	74	6.51E-12	2.0649E-09	
bta04360	Axon guidance	66	1.81E-10	2.86638E-08	

bta05205	Proteoglycans in cancer	69	6.37E-09	6.73064E-07
bta05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	32	2.9E-07	1.69379E-05
bta04810	Regulation of actin cytoskeleton	66	3.18E-07	1.69379E-05
bta04015	Rap1 signaling pathway	67	3.66E-07	1.69379E-05
bta05414	Dilated cardiomyopathy (DCM)	38	4.12E-07	1.69379E-05
bta05410	Hypertrophic cardiomyopathy (HCM)	36	4.77E-07	1.69379E-05
bta04010	MAPK signaling pathway	84	4.81E-07	1.69379E-05
bta04512	ECM-receptor interaction	35	5.96E-07	1.89009E-05

Figure 1. Differentially expressed genes (DEGs). **(A)** Expressed gene number in two groups based on the threshold of FPKM value > 1 . **(B)** Volcano plot for up/down-regulated DEGs. **(C)** Cluster of averaged transformed FPKM values for all DEGs in three replicates of the two groups. Note: The color from blue to red indicates the gene expression levels from low to high after the transform of $\log_{10}(\text{FPKM} + 1)$.

Figure 2. Experimental validations of selected **(A)** 9 up-regulated differentially expressed genes (DEGs) and **(B)** 10 down-regulated DEGs by qRT-PCR method. Note: The bars in the histograms indicate the standard errors of expression levels in three replicates.

Figure 3. Scatter plot for significant GO terms of 2565 differentially expressed genes (DEGs). Note: Only top 20 significant GO terms were visualized.

Figure 4. Scatter plot for significant KEGG pathways of 2565 differentially expressed genes (DEGs). Note: Only top 20 significant GO terms were visualized.

Figure 5. Network plot of protein-protein interactions (PPIs) for (A) up-regulated differentially expressed genes (DEGs) and (B) down-regulated DEGs. Note: Node size and node color indicate the degree and betweenness centrality, respectively.

Supplementary table 1. The distribution of expected number of fragments per kilobase of transcript sequence per millions base pairs sequenced among six samples.

Supplementary table 2. The primers of 19 selected DEGs for qRT-PCR .

Supplementary figure1. Scatter plots of correlations for three replicates of DMSO (control) and RSV (case) groups. Note: R^2 indicates the Pearson correlation coefficient.

Supplementary file 1. All up-regulated and down-regulated differentially expressed genes (DEGs) and the selected top 600 DEGs with string scores for protein-protein interactions (PPIs) analysis.

Supplementary file 2. GO term and pathway enrichment analysis of 2656 differentially expressed genes (DEGs) (both adjusted P -value < 0.05 and adjusted P -value < 0.0001).

Abbreviations

ARPC3: Actin related protein 2/3 complex subunit 3

CDKN1A: Cyclin-dependent kinase Inhibitor

COL3A1: Collagen type III alpha 1 chain

COL5A1: Collagen type V alpha 1 chain

COL6A2: Collagen type VI alpha 2 chain

CRABP1: Cellular retinoic acid-binding protein 1

CRABP2: Cellular retinoic acid-binding protein 2

CYP51A1: Cytochrome P450, family 51, subfamily A, polypeptide 1

DEG: Differentially expression gene

DM: Differentiation medium

DMEM: Dulbecco's modified Eagle's medium

DMSO: Dimethyl sulfoxide

EGCG: Epigallocatechin-3-gallate

EIF4E: Eukaryotic translation initiation factor 4E

F13A1: Coagulation factor XIII A chain

FC: Fold change

FDR: False discovery rate

FPKM: Fragments per kilobase of transcript sequence per millions base pairs sequenced

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

GM: Growth medium

GO: Gene Ontology

HMGCS1: 3-hydroxy-3-methylglutaryl-CoA synthase 1

ITGAV: Integrin subunit alpha V

ITGB1: Integrin subunit beta 1

ITGB5: Integrin subunit beta 5

LDH: Lactate dehydrogenase

MET: MET proto-oncogene receptor tyrosine kinase

MRF: Myogenic regulatory factor

mRNA: Messenger RNA

ORC1: Origin recognition complex subunit 1

PCC: Pearson correlation coefficient

PDGFRA: Platelet derived growth factor receptor alpha

PIK3R2: Phosphoinositide-3-kinase regulatory subunit 2

PLTP: Phospholipid transfer protein

PPI: Protein-protein interaction

qRT-PCR: Quantitative reverse transcription polymerase chain reaction

RAI1: Retinoic acid induced 1

RAI2: Retinoic acid induced 2

RAI14: Retinoic acid induced 14

RARA: Retinoic acid receptor alpha
RARB: Retinoic acid receptor beta
RARG: Retinoic acid receptor gamma
RARRES2: Retinoic acid receptor responder 2
RBPI: Retinol binding protein 1
RBP4: Retinol binding protein 4
RDH10: Retinol dehydrogenase 10
RDH11: Retinol dehydrogenase 11
RNAseq: RNA sequencing
RSV: Resveratrol
SDC3: Syndecan 3
THBS3: Thrombospondin 3

Author information

Affiliations

No. 22 Xinong Road, College of Animal Science and Technology, Northwest A&F University, Shaanxi Key Laboratory of Molecular Biology for Agriculture, 712100, Yangling, Shaanxi, China

Dan Hao, Xiaogang Wang, Xianyong Lan, Yongzhen Huang and Hong Chen

Quantitative Genomics, Bioinformatics and Computational Biology Group, Department of Applied Mathematics and Computer Science, Technical University of Denmark, Richard Petersens Plads, Building 324, 2800, Kongens Lyngby, Denmark

Dan Hao, Xiao Wang and Haja Kadarmideen

Department of Molecular Biology and Genetics, Aarhus University, 8000 Aarhus C, Denmark

Dan Hao and Bo. Thomsen

Contributions

DH performed all the experiments with the help of XGW. XGW performed qRT-PCR experiments. DH and XW analyzed the data and drafted the manuscript. BT, XW and HNK improved the manuscript. XYL and YZH helped to collect the bovine samples for the experiment. HC conceived and designed the experiments. #DH and #XGW contributed equally to this manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Data availability

Not applicable.

Ethical approval and informed consent

All the animal procedures were carried out according to the protocols approved by the College of Animal Science and Technology, Northwest A&F University, China. All the experimental animals were approved by the Institutional Animal Care and Use Committee in the College of Animal Science and Technology, Northwest A&F University, China.

Significant GO terms of 2565 differentially expressed genes (DEGs)

Significant pathways of 2565 differentially expressed genes (DEGs)

A Protein-protein interactions (PPIs) for up-regulated differentially expressed genes (DEGs)

B Protein-protein interactions (PPIs) for down-regulated differentially expressed genes (DEGs)

Highlight

1. Primary myoblasts treated with RSV and mRNA sequencing
2. Differentially expressed genes (DEGs)
3. Significant GO terms and significant KEGG of DEGs

4. Network of PPIs for DEGs.