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Original Research Article

Transcriptome analysis of adipose tissue and muscle of Laiwu and Duroc pigs

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ABSTRACT

Fat content is an important trait in pig production. Adipose tissue and muscle are important sites for fat deposition and affect production efficiency and quality. To regulate the fat content in these tissues, we need to understand the mechanisms behind fat deposition. Laiwu pigs, a Chinese indigenous breed, have significantly higher fat content in both adipose tissue and muscle than commercial breeds such as Duroc. In this study, we analyzed the transcriptomes in adipose tissue and muscle of 21-d-old Laiwu and Duroc piglets. Results showed that there were 828 and 671 differentially expressed genes (DEG) in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT), respectively. Functional enrichment analysis showed that these DEG were enriched in metabolic pathways, especially carbohydrate and lipid metabolism. Additionally, in the longissimus muscle (LM) and psoas muscle (PM), 312 and 335 DEG were identified, demonstrating enrichment in the cell cycle and metabolic pathways. The protein–protein interaction (PPI) networks of these DEG were analyzed and potential hub genes were identified, such as *FBP1* and *SCD* in adipose tissues and *RRM2* and *GADL1* in muscles. Meanwhile, results showed that there were common DEG between adipose tissue and muscle, such as *LDHB*, *THRSP*, and *DGAT2*. These findings showed that there are significant differences in the transcriptomes of the adipose tissue and muscle between Laiwu and Duroc piglets ($P < 0.05$), especially in metabolic patterns. This insight serves to advance our comprehensive understanding of metabolic regulation in these tissues and provide targets for fat content regulation.

1. Introduction

Fat content is an important trait in pig production. Adipose tissue is the primary site for fat deposition, including subcutaneous and visceral adipose tissues. The fat content in adipose tissue affects meat production rate and production efficiency (Wood et al., 2008). Muscle is the secondary important site for fat deposition and fat composition and content in muscle are important indicators of meat quality (Hausman et al., 2009; Wood et al., 2008). For the best production efficiency and quality, we need to regulate fat content in both adipose tissue and muscle, therefore understanding the mechanisms behind fat deposition in these tissues is a vital issue.

For the past decades, selective breeding has been applied to increase the growth rate and meat percentage to maximize production efficiency. As a result, available commercial breeds such as Duroc pigs exhibit low meat quality due to low fat content (Zhao et al., 2021). Compared to the lean-type breeds, the Chinese

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indigenous breed Laiwu pig has higher meat quality. The fat content in Laiwu muscle can reach 10%, significantly higher than Duroc pigs (Tang et al., 2020), and the whole-body fat content of Laiwu pigs can reach 40%, again significantly higher than Duroc pigs (20%). The excessive fat in Laiwu pigs mainly deposits in subcutaneous and visceral adipose tissues, which significantly reduces the lean meat rate. Therefore, the high fat content in adipose tissues of Laiwu pigs lowers production efficiency and has become a vital limitation for its application in production.

For the best production efficiency and quality, we need to regulate the fat content in both adipose tissue and muscle. Therefore, a comprehensive understanding of the mechanisms behind fat deposition in these tissues is in need. Laiwu and Duroc pigs have presented with significantly different fat accumulation capacities in both adipose tissue and muscle, thereby providing ideal models for studying the mechanism of fat deposition. In this study, we investigated the transcriptomes of the adipose tissue and muscle in 21-d-old Laiwu and Duroc piglets, and our results showed significant differences between breeds, especially in carbohydrate and lipid metabolism pathways.

2. Materials and methods

2.1. Animal ethics statement

All experiments complied with the Guide for the Care and Use of Laboratory Animals of South China Agricultural University, Guangzhou, China (Permit Number: 2022f125) and complied with the ARRIVE guidelines.

2.2. Animals and sample collections

A total of 12 male piglets at 21 d old were utilized for this study, with 6 each from Duroc and Laiwu pigs. All piglets were nursed by sows until they were 21 d old and were fasted overnight before sacrifice for tissue sample collection. Pre-slaughter weights of 12 pigs are shown in Table S1. Four tissues were collected from these pigs: subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), longissimus muscle (LM), and psoas muscle (PM). SAT samples were collected at the first rib location, and VAT samples were from the greater omentum. Samples were immediately placed in liquid nitrogen for snap freezing and subsequently stored at −80 °C. Total RNA was extracted by the TRIzol method (Invitrogen, USA).

2.3. RNA-Seq analysis

The RNA was processed to purify mRNA, synthesize cDNA, and prepare the library for paired-end sequencing using the Illumina Novaseq 6000 platform (Novogene Biotechnology Co., Ltd, Beijing, China). After obtaining raw sequencing reads, quality control was performed using fastp software (version 0.22.0) (Ramírez et al., 2016). After quality filtering, we obtained an average of approximately 28 million high quality clean reads for each sample. Subsequently, the HISAT2 software (version 2.1.0) was employed to build an index of the susScr11 (Sus scrofa 11.1) reference genome, subsequently the clean data were aligned to this reference genome (Kim et al., 2015), resulting in an average alignment rate of 95.99%. Among the mapped reads, the average percentages of uniquely mapped reads and multiply mapped reads across all samples are 89.64% and 3.25%, respectively. These statistics suggested that the sequencing quality is sufficiently high to proceed with further quantitative analysis. StringTie (version 2.1.6) was used for transcript assembly and quantification (Pertea et al., 2016). Gene expression was normalized using transcripts per million (TPM) values. Low-expressed genes, with TPM values less than 0.1 in 20% of the samples, were filtered out. The filtering results are shown in Table S2. The remaining genes' read counts were then inputted into the edgeR (version 3.38.4) to compare the differential expression among the groups (Robinson et al., 2010). Afterward, genes meeting the criteria of $P < 0.05$ and $|\log2\text{fold change (FC)}| > 1$ were designated as differentially expressed genes (DEG). The R package VennDiagram was used to identify the overlapping DEG among them (https://cran.r-project.org/web/packages/VennDiagram/index.html).

2.4. Enrichment analyses

To further understand the function of DEG, gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) function annotations were conducted using the DAVID website (https://david.ncifcrf.gov/) (Jiao et al., 2012). Principal components analysis (PCA), volcano plots for DEG distribution, and functional annotation analysis were visualized using R packages.

2.5. Protein–protein interaction (PPI) network and module analysis

Based on KEGG pathway analysis, we performed PPI analysis of DEG in the identified significant pathways using the STRING-db server with an interaction score of 0.4 (v12.0; https://version-12-0.string-db.org/). After constructing the network, the CytoHubba plugin (v0.1) of Cytoscape software (v3.10.0) was used to obtain the top 10 ranked genes based on the maximal clique centrality (MCC) method (Shannon et al., 2003) to predict the hub genes. Network visualization was performed in Gephi (v0.10.1) software (Bastian et al., 2009).

3. Results

3.1. Transcriptome of subcutaneous adipose tissues

In SAT, solo-PCA analysis using the conventional PCA architecture revealed a clear separation between the Duroc and Laiwu piglets indicating significant differences in their transcriptomes (Fig. S1). Differential gene expression analysis showed 828 DEG, and among these genes, 463 were down-regulated in Laiwu piglets, while 365 were up-regulated (Fig. 1A).

To clarify the functions of the identified DEG, GO and KEGG enrichment analyses were performed (Fig. 1B and C). Results showed that DEG were most significantly enriched in ATP binding (GO:0005524) and metabolic pathways (ssc01100), suggesting that the metabolic pattern was significantly different between 21-d-old Laiwu and Duroc SAT. Protein–protein interaction networks were then constructed for the DEGs in these pathways (Fig. 1D). In the PPI networks, we identified seven candidate genes predicted as hubs in the network, which were enolase 3 (*ENO3*), lactate dehydrogenase B (*LDHB*), aldolase, fructose-bisphosphate c (*ALDOC*), fructose-bisphosphatase 1 (*FBP1*), phosphofructokinase polypeptide X (*PFKM*), phosphoglycerate mutase 2 (*PGAM2*) and transketolase like 2 (*TKTL2*). *ENO3*, *PGAM2*, *FBP1*, *PFKM*, and *TKTL2* exhibited lower expression levels in Laiwu SAT than Duroc. *LDHB* and *ALDOC* were higher in Laiwu SAT. These hub genes were involved in carbohydrate metabolism pathways, suggesting a significant difference in metabolic patterns between Laiwu and Duroc in subcutaneous adipose tissue.

Meanwhile, we found that cell death-inducing DFFA-like effector c (*CIDEC*), which is a lipid droplet fusion and lipid storage regulator (Nishino et al., 2008), was significantly higher in Laiwu SAT than in Duroc. Fatty acid synthesis genes such as stearoyl-CoA desaturase (*SCD*) and elongation of long-chain fatty acids family

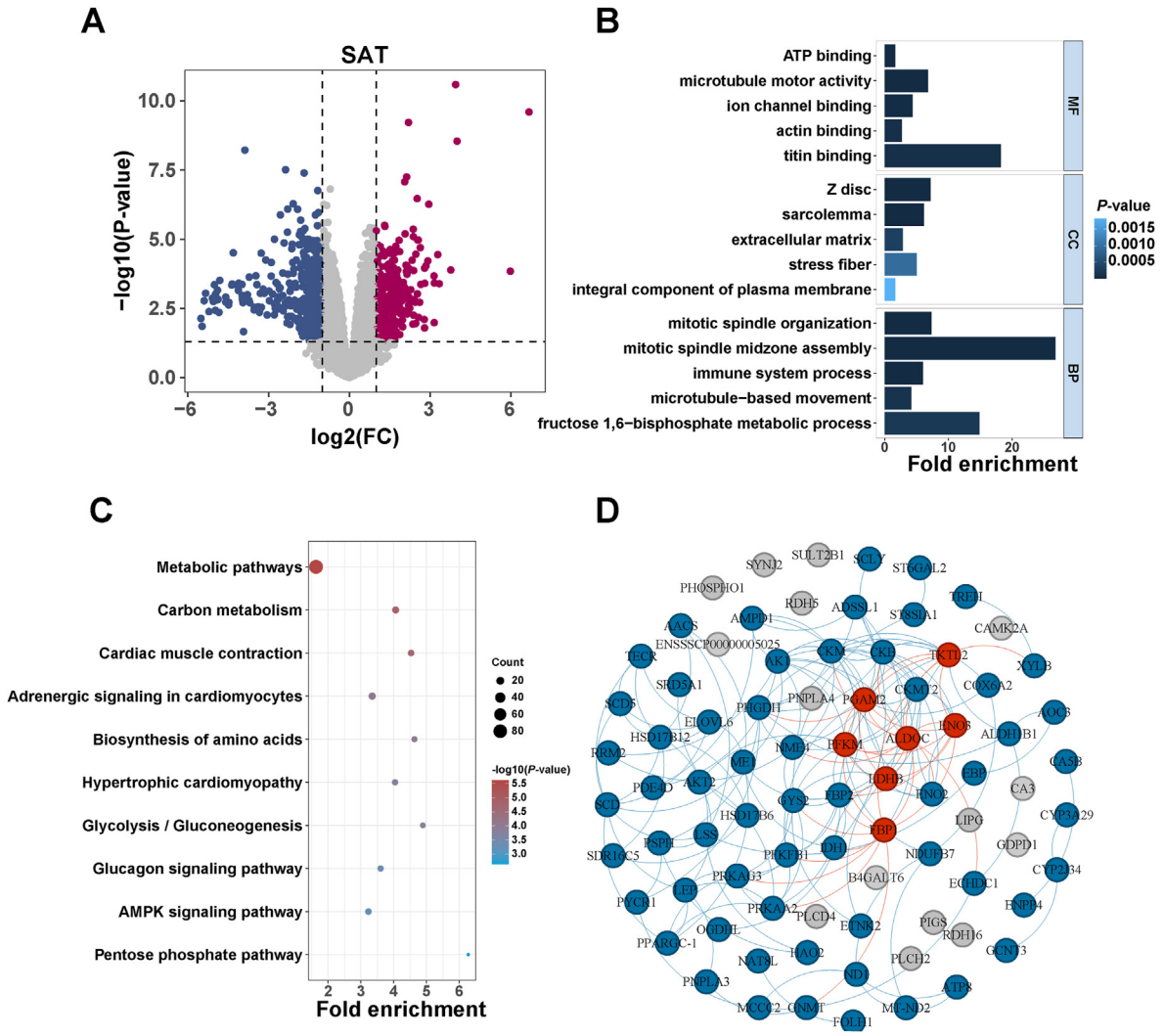

Fig. 1. Transcriptome analysis of subcutaneous adipose tissue (SAT) in Laiwu ($n = 6$) and Duroc ($n = 6$) pigs. (A) The volcano map of differentially expressed genes (DEG). The x-axis represents the fold change (FC) and the y-axis represents the *P*-value. Red dots indicate significantly up-regulated genes and blue dots indicate down-regulation. (B) The top five gene ontology (GO) terms. The GO terms are divided into three main domains: biological processes (BP), molecular functions (MF), and cellular components (CC). (C) The top ten Kyoto encyclopedia of genes and genomes (KEGG) pathways. The *x*-axis indicates the fold enrichment and the *y*-axis indicates the name of the term or pathway. Node color indicates significance and node size indicates the number of genes enriched in the pathway. (D) Protein−protein interaction (PPI) network diagram. Red nodes indicate predicted hub genes, blue nodes indicate genes with more than one connection, and gray nodes indicate genes with no connection. The color of the lines (red or blue) in the PPI network is determined by the color of the source node.

member 6 (*ELOVL6*) were also higher in Laiwu SAT than in Duroc, suggesting that lipid synthesis and storage were higher in Laiwu subcutaneous adipose tissue.

3.2. Transcriptome of visceral adipose tissues

In the visceral adipose tissue, PCA analysis showed that the first principal component accounts for 30% of the comparison between Duroc and Laiwu (Fig. S1B). A total of 671 DEG were identified. Among them, 418 genes were downregulated in Laiwu compared to Duroc pigs, while 253 genes were upregulated (Fig. 2A). The function enrichment analysis also revealed significant differences in metabolic pathways in VAT (Fig. 2B and C). The PPI network analysis suggested that DEG including squalene epoxidase (*SQLE*), collagen type VI alpha 5 chain (*COL6A5*), *COL4A4* and *COL13A1* might act as hubs in the network (Fig. 2D).

SQLE was significantly higher in Laiwu VAT than in Duroc VAT, which initiates cholesterol synthesis by introducing an epoxide group into the isoprenoid squalene, resulting in the formation of 2,3(S)-oxidosqualene. *SQLE* is one of the rate-limiting enzymes of cholesterol biosynthetic process and is involved in lipid droplet formation (Chua et al., 2020). Meanwhile, the upstream enzyme of *SQLE*, *HMGCR*, which is also a rate-limiting enzyme of de novo cholesterol biosynthesis (Endo, 1992), was also higher expressed in Laiwu VAT than in Duroc VAT. Besides hub genes, results showed that lipid metabolism genes such as monoacylglycerol O-acyltransferase 2 (*MOGAT2*), which catalyzes the synthesis of triacylglycerol (Yen et al., 2009), were higher in Laiwu VAT than in Duroc VAT. These results suggested that lipid metabolism such as cholesterol and triglyceride (TG) synthesis was significantly different in VAT.

Meanwhile, results showed that the expression of several collagens was significantly different in VAT between Laiwu and Duroc piglets. *COL4A2*, highly expressed in VAT, was significantly lower in Laiwu; *COL6A5*, *COL4A2* and *COL13A1* were also lower in Laiwu. Previous studies showed that these collagens may be involved in

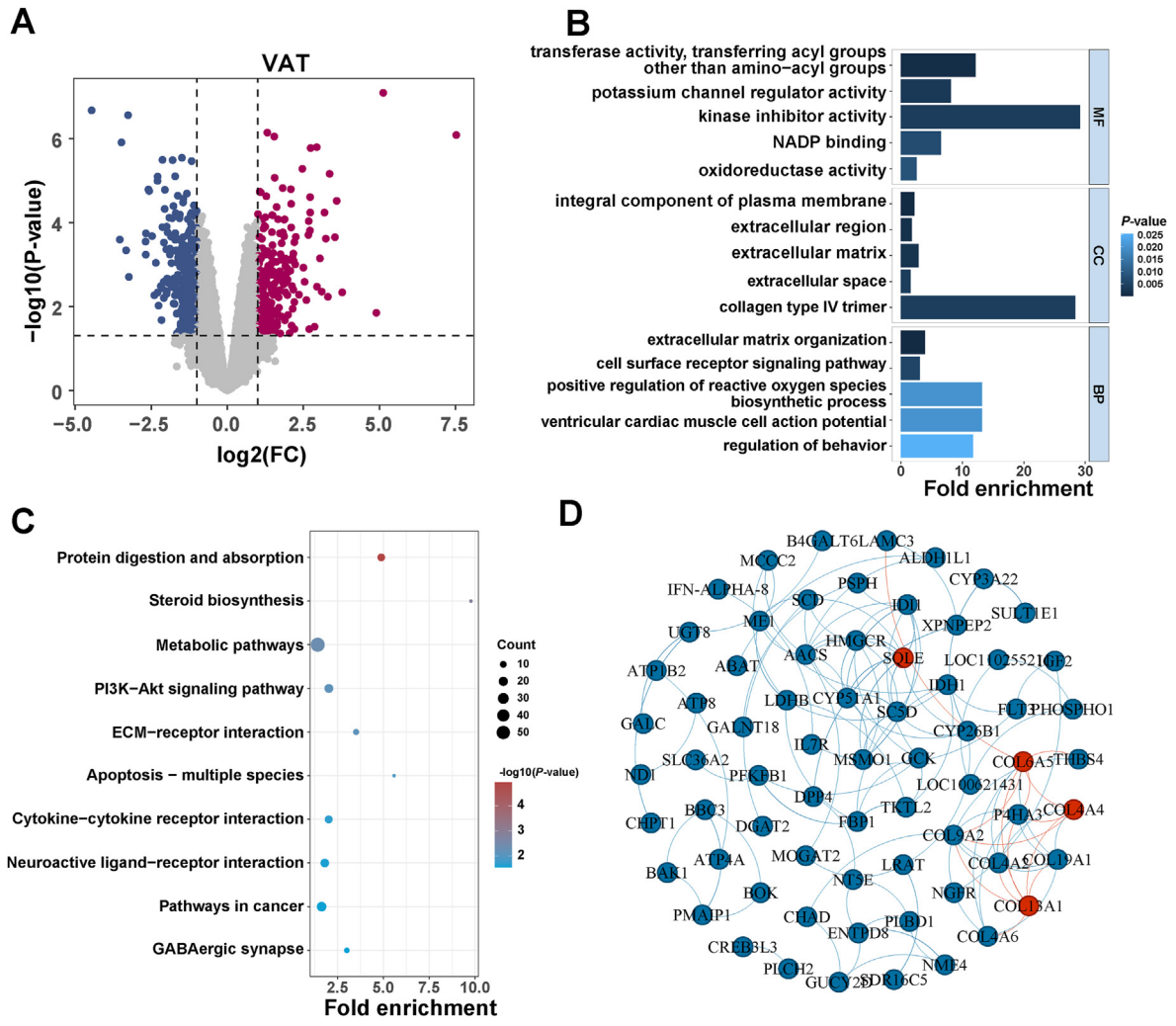

Fig. 2. Transcriptome analysis of visceral adipose tissue (VAT) in Laiwu ($n = 5$) and Duroc ($n = 4$) pigs. (A) Volcano plots of differentially expressed genes. The x-axis represents the fold change (FC) and the y-axis represents the *P*-value. (B) Gene ontology (GO) functional analysis. The GO terms are divided into three categories: biological processes (BP), molecular functions (MF), and cellular components (CC). (C) Kyoto encyclopedia of genes and genomes analyses. (D) Protein−protein interaction network.

lipid accumulation (Dong et al., 2021; Garritson et al., 2023; Kim et al., 2021). These findings indicated significant differences in metabolic pathways within the visceral adipose tissue of Laiwu and Duroc piglets, and the lipid metabolism difference was more significant in VAT.

We further analyzed the difference between SAT and VAT, and results showed that there were 596 and 439 DEG in SAT and VAT, specifically, 232 common DEG in both of them (Fig. S2A), suggesting that besides the above specific differences, there were also common differences in SAT and VAT between Laiwu and Duroc. Such as *FBP1*, a gluconeogenesis rate-limiting enzyme, showed lower expression in both SAT and VAT of Laiwu. Glycolysis regulator *PFKFB1* was higher in Laiwu adipose. These suggested that carbohydrate metabolism was a significant part of the metabolic differences between Laiwu and Duroc adipose tissue.

Results also showed common DEG in lipid metabolism. Diazepam binding inhibitor (*DBI*), a lipogenesis regulator in adipose tissue (Alquier et al., 2021), was high in both Laiwu SAT and VAT. Acetoacetyl-CoA synthetase (*AACS*), an adipogenic enzyme involved in the utilization of ketone bodies (Bergstrom, 2023), was also higher in Laiwu adipose tissues. The enzyme *SCD*, responsible for the synthesis long chain fatty acids, and diacylglycerol O-acyltransferase 2 (*DGAT2*), which catalyzes the final step of TG synthesis (Man et al., 2006), showed higher expression in Laiwu, suggesting a higher capacity for lipid synthesis. These results showed that there were both specific and common regulators affecting fat deposition in SAT and VAT, and would provide a basis for further understanding of different adipose tissues.

3.3. Transcriptome of longissimus muscle

In the LM, PCA analysis also showed clear separation (Fig. S1C), 312 differentially expressed genes were identified, with 140 genes being downregulated and 172 genes being upregulated (Fig. 3A). The DEG were then annotated for enrichment of GO terms and KEGG pathways (Fig. 3B and C), and results showed that metabolic pathways and cell cycle functions were significantly different. PPI networks analysis revealed *PPARGC1A, E2F1, MCM2* and *SCD* as hub genes (Fig. 3D).

Lipid synthetic enzyme *SCD* was higher in Laiwu LM; *PPARGC1A*, as well as *PPARA*, were down in Laiwu LM. Peroxisome proliferator-activated receptor-alpha (PPAR-α) is a ligand-activated transcriptional factor that has been demonstrated to modulate lipid metabolism by regulating fatty acid uptake and oxidation (Varga et al., 2011). *PPARGC1A* is a PPAR-γ coactivator and vital regulator in energy homeostasis (Lin et al., 2005). Aside from these hub genes,

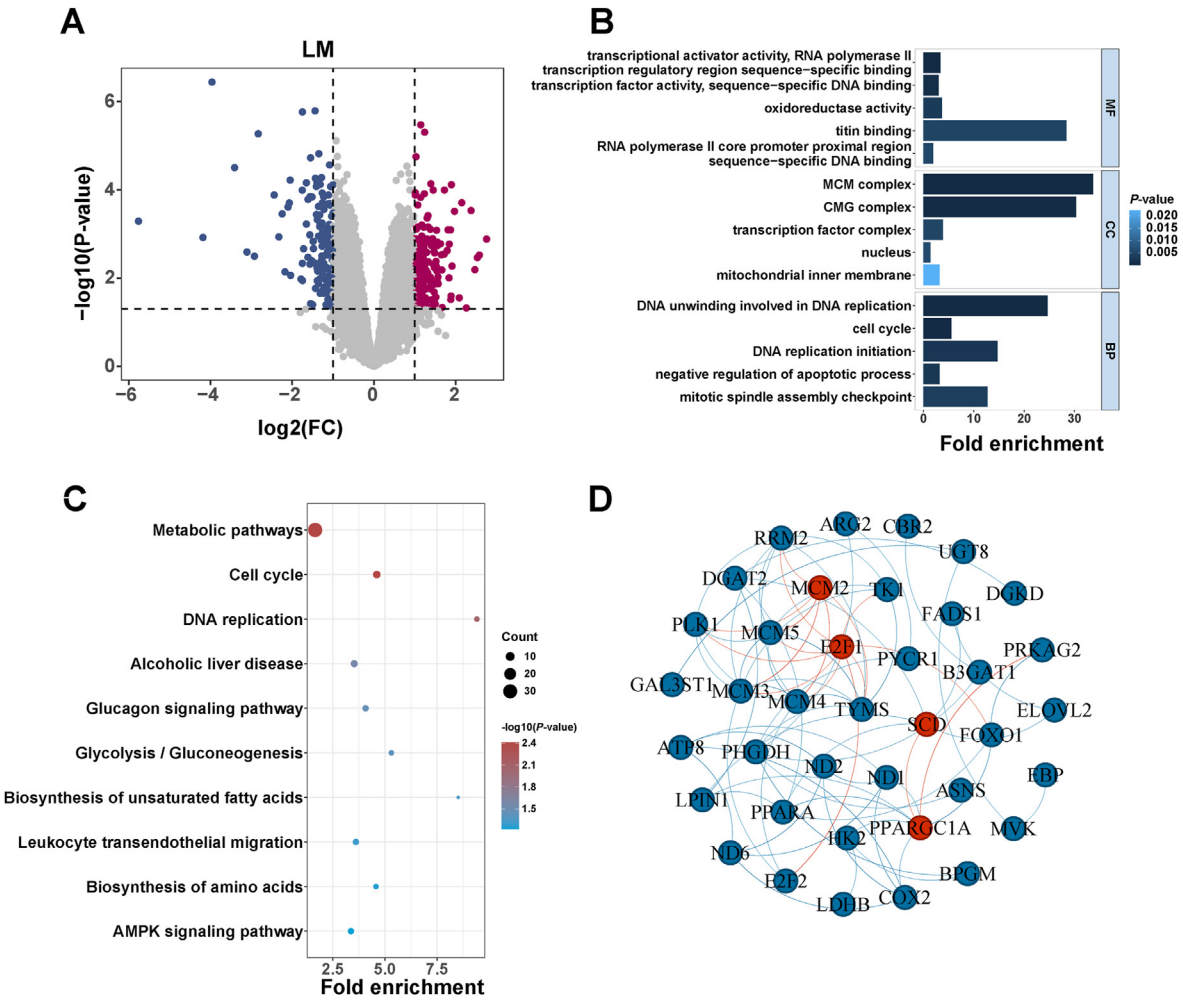

Fig. 3. Transcriptome analysis of longissimus muscle (LM) in Laiwu ($n = 6$) and Duroc ($n = 6$) pigs. (A) Volcano plots of differentially expressed genes (DEG). The x-axis represents the fold change (FC) and the y-axis represents the *P*-value. (B) Gene ontology (GO) and (C) Kyoto encyclopedia of genes and genomes (KEGG) analyses of DEG in LM tissue. The GO terms are divided into three categories: biological processes (BP), molecular functions (MF), and cellular components (CC). (D) Protein−protein interaction network.

DEG results showed that other lipid metabolism regulators, such as forkhead box O1 (*FOXO1*), were also lower in Laiwu LM, and reducing FOXO1 function may be important for promoting glucose utilization and lipid synthesis (Lundell et al., 2019; Puigserver et al., 2003). These results would suggest that there was a significant difference in lipid and glucose metabolism in LM between Laiwu and Duroc piglets.

E2F1, belonging to the E2F family of transcription factors which regulate the cell cycle (Maixner et al., 2020), was higher in Laiwu LM. Mini-chromosome maintenance protein (MCM) family members, MCM2, MCM3, MCM4, and MCM5, which function in DNA replication and as targets of E2F transcription factors (Leone et al., 1998; Tye, 1999), were all higher in Laiwu LM. Further studies are required to gain insights into the functions of these genes in the regulation of muscle lipid metabolism.

3.4. Transcriptome of psoas muscle

In the PM, the PCA and differential expression analysis revealed significant differences between Laiwu and Duroc (Fig. S1D), with 335 genes found to be differentially expressed, of which 100 were down-regulated and 235 up-regulated (Fig. 4A). Cell cycle and ATP binding was significantly different (Fig. 4B and C), a total of five candidate hub genes, *RRM2*, *CCNBI*, *CCNB2*, *CDK1* and *BUB1B* were identified in the PPI network in this pathway (Fig. 4D).

Mitotic cyclins *CCNB1* and *CCNB2*, and the cyclin-dependent kinase 1 (*CDK1*), were identified with higher expression in Laiwu PM. *BUB1B*, a member of the spindle assembly checkpoint protein family, was higher in Laiwu PM. Research has demonstrated that nonsense mutant *BUB1B* mice exhibit an accelerated onset of phenotypes such as skeletal muscle wasting and fat loss (Wijshake et al., 2012). Ribonucleotide reductase M2 (*RRM2*), which catalyzes the conversion of ribonucleotides into deoxyribonucleotides, is regulated by p53, and is essential for DNA synthesis and repair (D'Angiolella et al., 2012), was also higher. These hub genes showed that the cell cycle might be significantly different in PM.

We also analyzed the differences between longissimus and PM, there were 223 (in LM) and 246 (in PM) specific DEG and 89 common DEG (Fig. S2B). Cell cycle difference was notable in both muscle, such as *RRM2*, *TK1*, and *GTSE1* as common DEG. Cytosolic thymidine kinase 1 (*TK1*) is a widely known enzyme involved in cell cycle regulation and plays a vital role in nucleotide metabolism during DNA synthesis (Welin et al., 2004), and was higher in Laiwu muscle. *GTSE1*, G2 and s-phase expressed 1, which regulates the cell cycle by interacting with p53 (Xu et al., 2018), was also higher in Laiwu LM and PM.

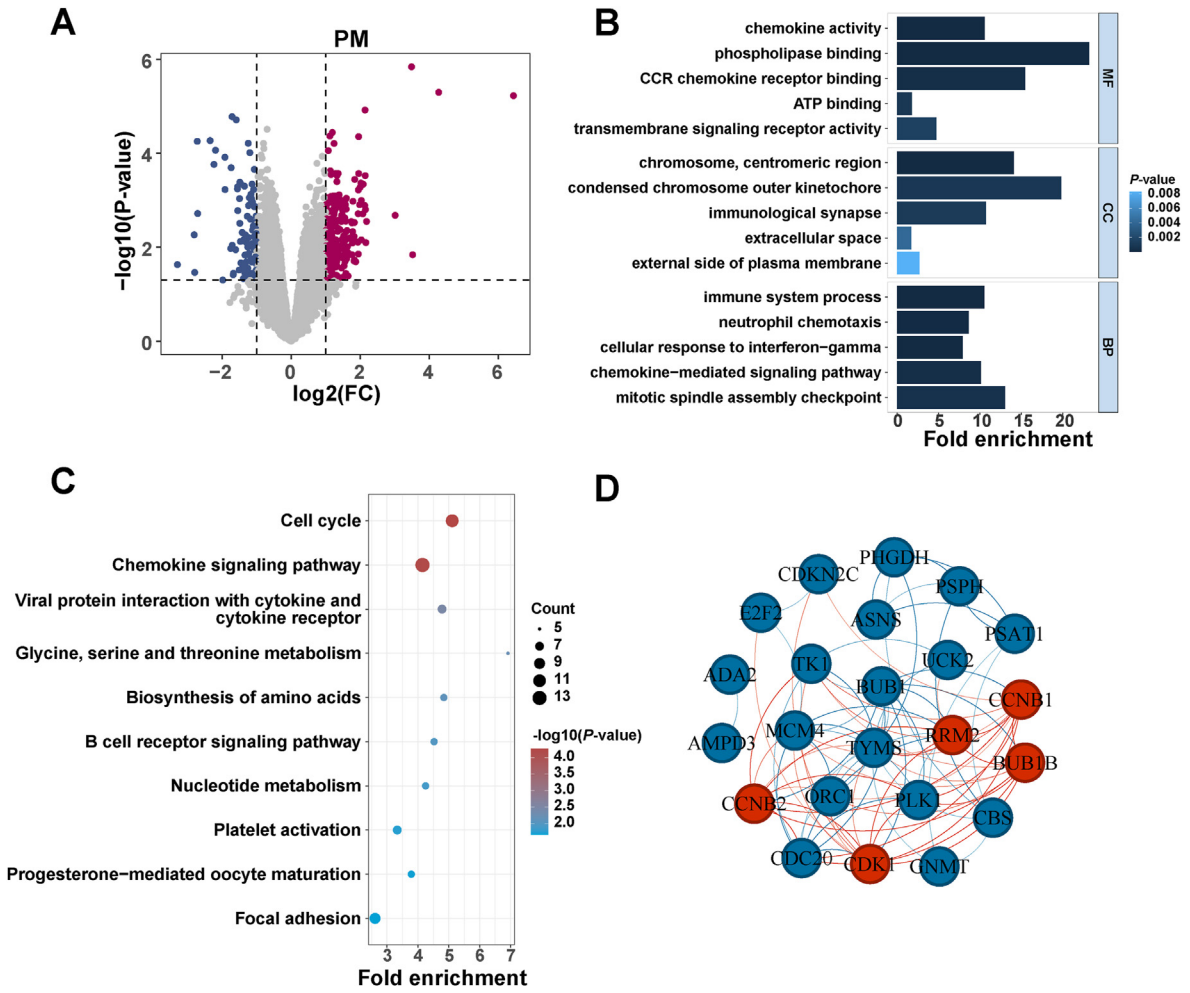

Fig. 4. Transcriptome analysis of psoas muscle (PM) in Laiwu ($n = 6$) and Duroc ($n = 6$) pigs. (A) Volcano plots analysis of the differentially expressed genes. The x-axis represents the fold change (FC) and the y-axis represents the *P*-value. (B) Gene ontology (GO) analysis. The GO terms are divided into three categories: biological processes (BP), molecular functions (MF), and cellular components (CC). (C) Kyoto encyclopedia of genes and genomes analysis (KEGG). (D) Protein–protein interaction network.

Energy metabolism differed less in PM than in LM; however, several common DEG were found, such as hexokinase 2 (*HK2*). HK2 is a key regulatory enzyme in glucose metabolism (Tan and Miyamoto, 2015), which was low in Laiwu muscle. Asparagine synthetase (ASNS), which regulates the tricarboxylic acid (TCA) cycle, glycolysis, amino acid metabolism, and energy metabolism by consuming aspartate, glutamine, and ATP (Huang et al., 2017; Lomelino et al., 2017), was higher. Glutamate decarboxylase like 1 (*GADL1*), a decarboxylase with a role in β-alanine and carnosine production which are important antioxidants, was high in Laiwu muscle. These results showed common differences in carbohydrate and lipid metabolism between Laiwu and Duroc muscle and provided potential targets for lipid content regulation.

3.5. Comparative analysis of muscle and adipose tissue transcriptomes

We further analyzed the transcriptome signatures in both adipose tissue and muscle, and results showed there were 14 common DEG (Fig. 5A; Table S3). They were *IGF2*, *ATP8*, *ND1*, *GPX1*, *DGAT2*, *LDHB*, *KLHL40*, *THRSP*, *RAB15*, *SLC26A10*, *LOC106506226*, *ENSSSCG00000018079*, *ENSSSCG00000002529* and *ENSSSCG00000048856* (Fig. 5B).

LDHB, a glucose metabolism pathway enzyme, was higher in both muscle and adipose tissues in Laiwu pigs. Thyroid hormone responsive (THRSP) is a lipogenic nuclear protein (Ahonen et al., 2022), which was more highly expressed in Laiwu adipose and muscle. Meanwhile, *DGAT2* was also higher in Laiwu, indicating a higher TG production after lipogenesis.

ND1, mt-tRNA and *ATP8* are all mitochondrial genes and results showed they were differentially expressed between Laiwu and Duroc. *ATP8* encodes the subunit of respiratory chain complex V, responsible for ATP production (Jonckheere et al., 2012). *ND1* encodes the subunit of NADH dehydrogenase and participates in mitochondrial oxidative phosphorylation (Sparks et al., 2005). These common DEG suggested that mitochondria function might be different between Laiwu and Duroc piglets; however, the exact differences need further investigation.

Glutathione peroxidase 1 (GPX1) is an antioxidant enzyme which is involved in the insulin signaling pathway in muscle. *GPX1* had higher expression in Laiwu muscle and adipose, suggesting a less sensitive insulin reaction since studies showed that overexpression of *GPX1* develops insulin resistance (McClung et al., 2004). However, whether this is a result of higher lipid content in adipose and muscle remains to be explained.

There was evidence suggesting that ras-related protein rab-15 (*RAB15*) acts as a negative regulator of endocytosis (Zuk and

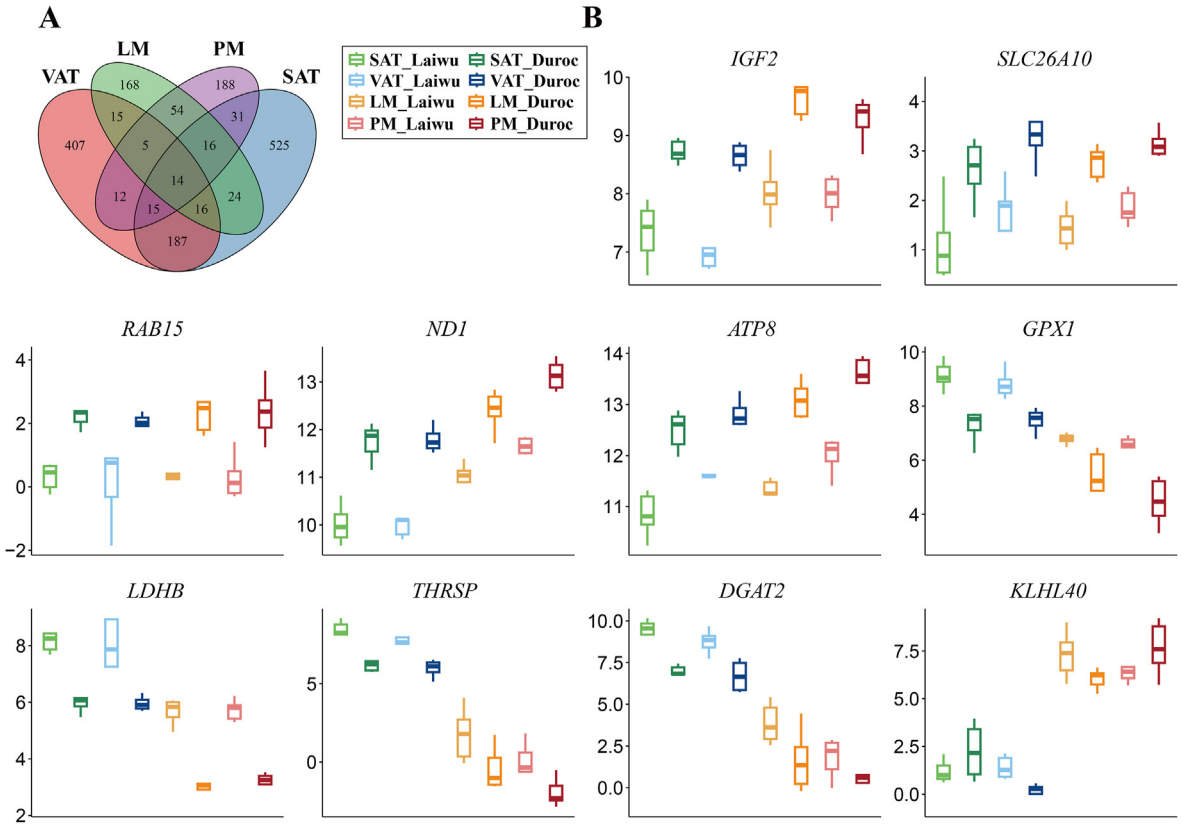

Fig. 5. Differentially expressed genes (DEG) identified in both adipose tissue and muscle of Laiwu and Duroc pigs. (A) Venn diagram of DEG. (B) Expression level distribution of ten genes with significant differences in both adipose tissue and muscle. Each color in the box plot represents a specific tissue, with the light and dark shades of the same color indicating Laiwu and Duroc, respectively. SAT = subcutaneous adipose tissue; VAT = visceral adipose tissue; LM = longissimus muscle; PM = psoas muscle. *IGF2* = insulin like growth factor 2; *SLC26A10* = solute carrier family 26 member 10; *RAB15* = ras-related protein rab-15; *ND1* = NADH dehydrogenase subunit 1; *ATP8* = mitochondrially encoded ATP synthase 8; *GPX1* = glutathione peroxidase 1; *LDHB* = lactate dehydrogenase B; *THRSP* = thyroid hormone responsive; *DGAT2* = diacylglycerol o-acyltransferase 2; *KLHL40* = Kelch like family member 40.

Elferink, 2000) and *RAB15* is a candidate gene associated with body weight in goats (Easa et al., 2022). *RAB15* expression was lower in Laiwu adipose and muscle, which might suggest that the cellular uptake of substances such as lipoproteins via endocytosis could be higher than in Duroc. *SLC26A10* was significantly lower in Laiwu, and there is evidence suggesting that *SLC26A10* is implicated in obesity (Kim et al., 2022).

Insulin like growth factor 2 (*IGF2*) was lower in Laiwu muscle and adipose tissue, which is related to muscle growth rate, heart size and fat accumulation in pigs (Van Laere et al., 2003). The remaining common DEG, *LOC106506226*, *ENSSSCG00000048856*, *ENSSSCG00000018079* and *ENSSSCG00000002529*, may be novel genes, whose functions need to be further investigated.

4. Discussion

Fat deposition is a vital factor that not just affects animal production efficiency and quality, but also has a significant impact on human health. Many efforts have been made to understand the mechanisms behind fat deposition in multiple tissues and organs. The Chinese indigenous pig breed Laiwu, which has excessive fat content in both muscle and adipose tissue has provided an excellent animal model for exploring the mechanisms behind fat deposition in these tissues (Wang et al., 2022; Xie et al., 2022). Here we comparatively analyzed the transcriptomes of adipose tissue and muscle in 21-d-old Laiwu and Duroc piglets to understand the potential regulation mechanisms at an early age. Our results have shown a clear separation between Laiwu and Duroc. A total of 828 and 671 DEG in SAT and VAT, respectively, and 321 and 335 in LM and PM, respectively were found, suggesting early up to 21 d there has been a significant difference in adipose tissue and muscle, and early intervention of fat accumulation would be needed in order to regulate Laiwu pig fat content.

Further analysis showed that the metabolic pattern in the high-fat-content model Laiwu pigs and lean model Duroc pigs had significant differences. Glucose metabolism, especially glycolysis, serves as the initial step in the metabolic network, which not only results in ATP synthesis but also regulates biosynthetic pathways such as fatty acid synthesis, nucleic acid synthesis, amino acid metabolism, and energy metabolism. Our results identified many DEG in the glucose metabolism pathway, such as *LDHB*, *PFKFB1*, and *FBP1*. LDHB is an enzyme catalyzing the interconversion of pyruvate and lactate (Chen et al., 2016), and there is evidence showing that *LDHB* was higher in Laiwu LM (Zhang et al., 2022), corresponding with our results which showed that *LDHB* was higher in both muscle and adipose tissues in Laiwu pigs, suggesting a higher capacity to convert lactate to lipid. *PFKFB1* belongs to the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase family, which plays a crucial role in glucose metabolism by producing fructose-2,6-bisphosphate (F26BP) and serves as a powerful activator of glycolysis (Okar et al., 2001), and was found to be higher in Laiwu adipose tissue. Additionally, *ALDOC* exhibited higher expression in Laiwu SAT, and studies have demonstrated that *ALDOC* plays a central role in fructolysis (Chang et al., 2018). This

evidence suggested that higher glycolysis and fructolysis would provide more energy substrates such as fructose, lactate, and even ketone bodies for downstream fatty acid and TG synthesis, leading to higher fat deposition. Meanwhile, *TKTL2*, a transketolase-like gene functioning in the pentose phosphate pathway (PPP), a multifunctional pathway which produces a reduced form of NADPH for lipid biosynthesis, and converts hexoses into pentoses to support nucleic acid synthesis (Qin et al., 2019), was lower in Laiwu adipose tissue, suggesting that differential glucose metabolism could have an impact on other synthetic pathways such as nucleic acid metabolism. Glycolysis genes *ENO3*, an enolase whose mutations have been associated with glycogen storage and glucose intolerance (Pei et al., 2006), and *PGAM2*, phosphoglycerate mutase which catalyzes the reaction of 3-phosphoglycerate to 2-phosphoglycerate in glycolysis with mutations to it causing glycogen storage disease (Adeva-Andany et al., 2016), were different between Laiwu and Duroc, suggesting the glycogen metabolism could also be affected. These findings emphasized the role of glucose metabolism on Laiwu fat deposition regulation, and it is notable that we may think more of carbohydrate nutrients to regulate lipid content in pig production.

Previous studies showed that lipid synthesis was higher in adipose tissue of fat type pig breeds (Albuquerque et al., 2020; Pan et al., 2022; Wang et al., 2017). Results showed that *FASN* expression strongly correlated with intramuscular fat (IMF) content in Laiwu pigs (Wang et al., 2020). Our results also showed that the capacity of lipid synthesis in Laiwu adipose tissue and muscle was high. Laiwu pig adipose tissue and muscle could have a higher capacity for de novo fatty acid synthesis, since higher expression of lipogenic genes such as *THRSP*. Evidence might suggest that Laiwu adipose and muscle could utilize a variety of substrates such as salvaged lactate and ketone body to produce lipids. Meanwhile, long chain fatty acid synthesis and TG synthesis in Laiwu pigs could also be high, since higher expression of *SCD*, *DGAT2*, and *ELOVLs* (Hausman et al., 2009; Man et al., 2006).

The antioxidative capacity seemed to be different in Laiwu adipose tissue and muscle since our results showed several oxidative stress-related DEG. Superoxide dismutase 3 (*SOD3*) is related to oxidative stress (Wang et al., 2018), and was lower in Laiwu VAT and LM. Carbonic anhydrase III (*CA3*), a pivotal enzyme involved in the reversible hydration of carbon dioxide and with antioxidative properties (Räisänen et al., 1999), is much higher in Laiwu adipose. Paraoxonase 1 (PON1), an antioxidant enzyme (Costa et al., 2005), was higher in Laiwu adipose tissues. Glutamate decarboxylase like 1 (GADL1), an enzyme for producing important antioxidants (Mahootchi et al., 2020), was higher in Laiwu muscle. Previous studies showed that *GADL1* deletion altered energy and lipid metabolism (Mahootchi et al., 2020). These results may suggest a higher antioxidative capacity, which could be an important contributor to the high fat accumulation, in Laiwu pigs.

Mitochondria function seems to be different in adipose tissue and muscle between Laiwu and Duroc. As the energy production center of cell, mitochondria function is vital for energy balance. Not only were mitochondria function regulators such as *PPARGC1A* differentially expressed, results also showed that several mitochondrial genes were significantly different in both adipose tissue and muscle, such as *ND1*, *ATP8*, and mt-tRNA, which were lower in all adipose tissue and muscle in Laiwu pigs. Meanwhile, mitochondrial genes, including mt-rRNA, *ND2*, *COX2*, *ND6*, etc., were specifically different in some of the four tissues (Table S4). These genes encode important molecules in the mitochondrial process of oxidative phosphorylation (OXPHOS) and ATP production, and results showed that they were expressed at lower levels in Laiwu pigs. This evidence suggested that the mitochondria of Laiwu piglets might be significantly different in adipose tissue and muscle than in Duroc; however, whether mitochondria functions such as OXPHOS and energy production were lower would need further studies. The explanation of these low expressed mitochondrial genes might be complex, but the mitochondrial function in Laiwu adipose tissue and muscle is worth further investigation.

Besides common differences, our results have also shown specific differences in both adipose tissue and muscle. The fat content in pig muscle, usually called IMF, is composed of intracellular fat and intercellular fat which mainly refers to intracellular adipose tissue. It is worth noticing that our results showed that the read counts of adipose-specific markers such as adiponectin (*ADIPOQ*) and perilipin 1 (*PLIN1*) were significantly low in muscle transcriptome results, suggesting that there was little adipose in 21-d-old pig muscle samples. The metabolic differences shown by our results have indicated that there could be a genetic determination on muscle intracellular lipid production and accumulation. Our results showed that DEG such as *THRSP* and *DGAT2* indicated a higher capacity for lipid synthesis in Laiwu muscle. THRSP, a lipogenic nuclear protein that is highly associated with lipid metabolism and fat deposition in multiple animals (Polasik et al., 2021; Yao et al., 2016), was significantly higher in Laiwu muscle. These results could provide information for intracellular muscle fat content regulation and for muscle metabolism and function. Meanwhile, results showed no significant difference in muscle lipid absorption in Laiwu pigs, suggesting that ectopic fat deposition could only be responsible for a fraction of Laiwu pig muscle lipid content.

Cell cycle regulation was significantly different in muscle between Laiwu and Duroc piglets. The hub genes *E2F1* in LM, *CCNB1*, *CCNB2*, and *CDK1* in PM, and *RRM2* in both LM and PM, which are vital cell cycle regulators, exhibited higher expression levels in Laiwu piglets; however, 21-d-old Laiwu piglets body weight was lower than that of Duroc piglets. Development rate is a vital factor for production efficiency, and not only do 21-d-old piglets exhibit a lower development rate, but also the overall production time of Laiwu pigs is lower than commercial breeds such as Duroc. We further analyzed this aspect and found that *IGF2* was lower in Laiwu adipose tissue and muscle, suggesting a lower growth rate (Lau et al., 1994; Van Laere et al., 2003). Moreover, our results showed DEG in adipose tissue that might affect animal growth rate. For example, *COL13A1* exhibited lower expression in Laiwu adipose than in Duroc, and there were studies showed that the absence of *COL13A1* could cause feeding problems and low growth rate (Härönen et al., 2017; Rodríguez Cruz et al., 2019). IGFALS gene expression was lower in both adipose tissues of Laiwu pigs, and studies showed that down regulation of *IGFALS* in mice caused growth retardation (Amuzie and Pestka, 2010). The low development phenotype of Laiwu pigs would be the regulatory result of both adipose tissue and muscle, and further investigation is needed. We can see that there have been significant differences between 21-d-old piglets of the two breeds, and the DEG we identified could provide useful information for future studies.

Notably, studies are showing that identified hub genes such as *E2F1* and *MCM2* might be involved in lipid metabolism regulation. Studies showed that *E2F1* expression was elevated in obese adipocytes (Maixner et al., 2020) and *E2F1* acted as a stimulator during adipogenesis (Fajas et al., 2002). *MCM2* deficient mice showed muscle weakness and loss of adipose tissue (Pruitt et al., 2007). These DEG suggested differences in cell cycle and DNA replication, which could also affect energy metabolism and lipid accumulation.

Besides the common DEG of adipose and muscle, our results showed many adipose-specific DEG that could provide targets for adipose lipid content regulation. It is worth noticing that the apoptosis was significantly different in adipose between the Laiwu and Duroc breeds. BAK1, a member of the BCL2 protein family,

serves the function of promoting apoptosis (Chittenden et al., 1995), and was lower in Laiwu VAT. BBC3, a member of the Bcl-2 BH3-only family and a potent inducer of apoptosis that is upregulated by p53 (Han et al., 2001), was also lower. BOK, when overexpressed, initiates apoptosis and shares its highest amino acid sequence similarity with *BAK* (Llambi et al., 2016), but its level was lower. BIRC5 encodes survivin, a protein belonging to the apoptosis-inhibiting family, that inhibits apoptosis and facilitates cell division (Li et al., 1998), and was higher in Laiwu SAT. These results suggested differences in apoptosis of adipocytes which could be an important factor affecting lipid accumulation, and targeting adipocyte apoptosis could also be a strategy to decrease fat accumulation in adipose tissues.

Fat content is also a vital factor that affects human health. The pig model has become an emerging method that can provide a comprehensive understanding of metabolic control. Our results showed that the differences in adipose tissue and muscle between fat-type and lean-type animals appear as early as 21-d-old, and the difference between metabolic patterns was the most significant. Our results indicated that there could be a genetic determination in intercellular glucose metabolism, lipid production, and accumulation in adipose tissue and muscle, in addition to lipid absorption. Metabolic patterns could be different in multiple pathways, not just glucose and lipid metabolism, but also amino acid metabolism, mitochondria function, and antioxidative capacity, etc. Our results have shown many DEG and many clues for a comprehensive understanding of lipid accumulation, and further investigations will be needed to validate whether they function as causal factors. Some non-coding RNA were identified as DEG such as lncRNA and their roles in fat deposition could be further studied.

5. Conclusion

By analyzing the transcriptomes of 21-d-old male Laiwu and Duroc adipose tissue and muscle, we found that there were significant differences, especially in metabolic pathways. Our results showed specific DEG such as *FBP1* and *SCD* in adipose tissue and *RRM2* and *GADL1* in muscle. We also found genes that were differentially expressed in both adipose tissue and muscle, such as *LDHB*, *THRSP* and *DGAT2*. These results suggested that Laiwu adipose tissue and muscle could have specific metabolic patterns, particularly in carbohydrate metabolism, to result in a high lipid synthetic capacity. Our results would provide useful information for future investigation to understand and regulate fat deposition in adipose tissue and muscle.

Author contributions

Lin Zhang: Supervision, Conceptualization, Writing-original draft, Writing-review & editing, Funding acquisition, Project administration, Resources. **Yulong Yin:** Supervision, Project administration. **Qingyan Jiang:** Supervision, Funding acquisition. **Jie Wu:** Data curation, Formal analysis, Writing-original draft, Writing-review & editing, Visualization. **Fangyuan Yu:** Investigation. **Zhaoyang Di:** Investigation. **Liwen Bian:** Investigation. **Jie Yang:** Methodology. **Lina Wang:** Data curation.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix supplementary data

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