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RESEARCH ARTICLE

Integrated metabolome, proteome, and transcriptome analysis explored the molecular mechanism of phosphoglycerate kinase 1 and pyruvate kinase M2 characterizing the postmortem meat quality

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Abstract

Phosphoglycerate kinase 1 (PGK1) and pyruvate kinase M2 (PKM2) have been identified as the postmortem meat quality biomarkers. However, the precise molecular mechanism through which they affect and regulate the development of meat quality remains unclear. In this work, the high- and low-activity groups (n = 10) were selected from 60 lamb muscles at 24 h postmortem based on the activity levels of PGK1 and PKM2. The metabolomic, proteomic, and transcriptomic analyses combined with deeply integrated multi-omics analysis were used to elucidate the mechanisms by which PGK1 and PKM2 characterize meat quality. The results indicated that glycolysis played a crucial role in regulating PGK1 and PKM2 activity at the metabolome, proteome, and transcriptome levels. In glycolysis pathway, we identified several key components closely related to PGK1 and PKM2 activity, including differential metabolites (adenosine triphosphate, adenosine diphosphate, glucose-6-phosphate, nicotinamide adenine dinucleotide phosphate, fructose-6-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, NAD^+ nicotinamide adenine dinucleotide, lactate, and pyruvate), different abundance proteins (lactate dehydrogenase B and fructose bisphosphate aldolase B), and differentially expressed genes (hexokinase and fructose-1,6-bisphosphatase 1). It was concluded that PGK1 and PKM2 may affect the formation of meat quality by regulating these critical substrates. Additionally, PGK1 and PKM2 could also affect the tricarboxylic acid cycle, oxidative phosphorylation, and muscle contraction in postmortem and then influence meat quality. This integrative omics study offers valuable insight into unraveling the molecular mechanisms underlying postmortem meat quality development.

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KEYWORDS
glycolysis, meat quality, multi-omics, phosphoglycerate kinase 1, postmortem muscle, pyruvate kinase M2

1 | INTRODUCTION

Meat quality not only has a significant impact on consumers' purchasing decisions and industry's economic performance but also on food waste and environmental pollution resulting from the deterioration of postmortem meat quality. Hence, comprehending the biological mechanisms governing the development of postmortem meat quality assumes paramount importance. Many physiological, biochemical, and metabolic changes occur during the postmortem transformation of muscle into meat, directly influencing and determining the postmortem meat quality (Matarneh et al., 2021). Among them, glycolysis, an essential energy metabolism pathway, plays a direct and influential role in the final meat quality development (Chauhan & England, 2018; Chen, L., Bai, Y., Everaert, N., Li, X., Tian, G., Hou.,C., & Zhang, D. 2019). Phosphoglycerate kinase 1 (PGK1) and pyruvate kinase M2 (PKM2) are the sole adenosine triphosphate (ATP)-generating enzymes involved in the glycolytic pathway, which can influence the rate of postmortem glycolysis (Hamm, 1977; Honikel, 2014). Our previous studies found that PGK1 and PKM2 could be identified as protein biomarkers to characterize postmortem meat quality (Huang, Blecker, et al., 2023; Huang, Zhang, et al., 2023). However, the molecular mechanisms by which PGK1 and PKM2 characterize the formation of postmortem meat quality remains unclear.

Over the last two decades, progress in biotechnology has given rise to high-throughput technologies, such as gene and RNA sequencing, proteomics, metabolomics, lipidomics, and more. These techniques enable the simultaneous examination of numerous molecules within individual biological specimens, providing a comprehensive perspective on bioprocess (Naba et al., 2016). Bioinformatics is consistently integrated with omics technologies to derive significant insights from intricate biological phenomena. In particular, meta-analysis of multiple omics datasets enables a comprehensive examination of the functions of biological molecules, the identification of coordinated mechanisms across different biological levels, and the screening of decisive genes, proteins, or metabolic processes (Misra et al., 2019). Integrated multi-omics analysis has been widely used in medical research to investigate disease biological indicators and their biological mechanisms (Zheng et al., 2023; Zhu et al., 2022). In the field of meat science, proteomics, transcriptomics, and metabolomics approaches are widely employed to study various aspects related to postmortem meat quality, such as the screening of markers for meat quality or spoilage, and the investigation of the underlying molecular mechanisms involved (Gu et al., 2023; Huang et al., 2020; Zhang et al., 2022). These technologies have significantly advanced our understanding of the molecular processes involved in postmortem meat quality development, providing valuable insights into meat preservation, spoilage detection, and enhancing meat product attributes. However, the integrated analysis of metabolomics, proteomics, and transcriptomics to reveal the molecular mechanisms of postmortem meat quality at different molecular levels has not been reported yet. Therefore, the application of integrative omics in meat science holds great promise for advancing the meat industry and improving consumer satisfaction.

The present study aimed to elucidate the molecular mechanisms insight on the characterization and regulation of postmortem meat quality by PGK1 and PKM2. Lamb meat samples with the differential PGK1 and PKM2 activity were selected at 24 h postmortem. Targeted metabolomics, quantitative proteomics, and transcriptomics were used to identify changes in the different activity of PGK1 and PKM2 at metabolome, proteome, and transcriptome levels. Additionally, a comprehensive multidimensional bioinformatics analysis revealed the molecular mechanisms by which PGK1 and PKM2 contribute to postmortem meat quality characterization. This work will provide extensive knowledge of the possible mechanisms involved in forming postmortem meat quality and offer scientific insights to reduce inferior meat while enhancing the production of high-quality meat.

2 | MATERIALS AND METHODS

2.1 | Samples collection

The study was performed following the guidance and under the approval of the Animal Welfare and Ethics Committee of the Institute of Food Science and Technology of Chinese Academy of Agricultural Sciences (ISFT2022-006). Sixty male Tan sheep aged 6–8 months with consistent feeding environments were selected from Ningxia Yanchi Tan Sheep Industry Development Group Co., Ltd. The sheep slaughter procedure was carried out in accordance with the identical protocol outlined in our earlier study (Huang et al., 2023). For 3 consecutive days, a total of 20 sheep were slaughtered each day. Within 30 min after bleeding, 60 left *Longissimus thoracis* (LT) were collected. The hot carcass weight of the collected samples was 21.29 ± 1.33 kg. Subsequently, all the samples were exposed to air and then wrapped with oxygen-permeable polyethylene film, which had an oxygen transmission rate of 10,600 $cm^3/(m^2\ 24\ h\ atm)$ and a moisture transmission rate of 68.5 $g/(m^2\ 24\ h)$. The muscle samples were stored in a chilling room (0–4°C) within the slaughterhouse. On-site measurements of shear force, cooking loss, a^* value, and R630/580 value were conducted at 24 h postmortem. Simultaneously, approximately 200 g samples were rapidly immersed in liquid nitrogen and then promptly moved to a freezer set at −80°C for subsequent index assessments.

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2.2 | Meat quality measurement

2.2.1 | a^* Value and R630/580

The measurement of a^* value and R630/580 was conducted using the same procedure outlined in our earlier study (Huang Blecker et al., 2023). Meat color was performed after 45 min of blooming at 4°C using of the Minolta CM-600D spectrophotometer (Konica Minolta Sensing Inc.). An aperture size of 8 mm with a D65 illuminant and a 10° standard observer was used in the whole experiment. The a^* value was determined by averaging readings from four randomly selected sites on the surface of each fresh cut sample, with deliberate avoidance of visible fat and connective tissue.

2.2.2 | Cooking loss and shear force

The method of cooking loss and shear force was described as Hopkins et al. (2010) with slight modifications. Muscle samples were weighed and recorded as W1 and then placed inside a cooking bag. The air inside the bag was evacuated to ensure close contact between the muscle surface and the bag. Lamb muscle samples were immersed in the water bath with a thermoregulator and a 1500 W heating element (HH-4, Weipinyiqi, Shenzhen, China) and cooked at 71°C for 35 min. Subsequently, samples were taken out and chilled in cold flowing water for 30 min. Surface moisture was wiped off with filter paper, and the samples were reweighed as W2. The cooking loss was determined using the subsequent equation:

$$\text{Cooking loss } (\%) = \frac{W1 - W2}{W2} \times 100\%$$

Samples were placed into a refrigerator set at 4°C overnight and then cut into cubes measuring 1.0 × 1.0 × 1.5 cm^3. Shear force was detected using a tender meter (C-LM4, Northeast Agricultural University, Harbin, China), with each sample subjected to 10 technical replicates.

2.3 | PKM2 and PGK1 activity

Pyruvate kinase activity and 3-phosphoglycerate kinase activity were determined using the assay kits (BC0545 and BC2255, Solarbio) as per the instructions provided by the manufacturer. The absorbance was measured using a microplate reader (Multimode Microplate Reader, Spark, Tecan). The enzymatic activity was presented as units per milligram of protein (U/mg protein).

2.4 | Targeted energy metabolomics

Energy metabolomics was performed using multi-reaction monitoring (MRM) technology. Briefly, 80 mg LT muscle was homogenized in 200 μL ultrapure water using a mechanical pestle. Subsequently, 800 μL of a methanol–acetonitrile solution (1:1, v/v) and 10 μL the internal standard (succinic acid-d6) were added, and the mixture was vortexed. The mixture underwent microtherm sonication for 30 min, with this process repeated twice. The solution was then preserved at −20°C for 1 h to obtain protein precipitation. The supernatant was gathered and subjected to freeze-drying after centrifugation (at 14,000 rcf and 4°C for 20 min). Ultimately, the supernatants were analyzed using the Agilent 1290 Infinity LC system in conjunction with the AB 5500 QTRAP mass spectrometer operating in the negative ion mode. This was followed by the process of identifying and quantifying the targeted metabolites.

2.5 | Proteomics

The method of tandem mass tag (TMT)-quantitative proteomics was the same as the description provided in a previous study (Huang Blecker et al., 2023). First, LT muscle was lysed in SDT buffer containing protease inhibitors to extract proteins. The protein content was assessed by the BCA Protein Determination Kit (Bio-Rad), and protein digestion was carried out using trypsin. Then, the resulting peptide digest from the sample underwent desalting using Sep-Pak C_{18} cartridges and was afterward vacuum-dried using a Speed Vac. Subsequently, the peptides were used in the subsequent TMT-10plex labeling-based proteomics analysis. The peptide samples labeled with tags were then segregated using the High pH Reversed-Phase Peptide Fractionation Kit (Thermo Fisher Scientific Inc.). Finally, the labeled peptides were analyzed using an LC–MS/MS system consisting of a Q Exactive mass spectrometer coupled with an EASY-nLC system.

2.6 | Transcriptomics

Transcriptomic—RNA sequencing (RNA-seq)—was conducted to discover the transcriptome changes in muscle samples with the different activities of PGK1 and PKM2. RNA was isolated from muscle samples by TRIzol reagent (Magen). The absorbance ratio of RNA samples at A260/A280 was measured using the Nanodrop ND-2000, followed by determining RNA integrity number value by Agilent Bioanalyzer 4150. First, oligo-magnetic beads were used to isolate mRNA from the total RNA. Subsequently, mRNA disintegration was performed using divalent cations at elevated temperatures in ABclonal's First Strand Synthesis Reaction Buffer. The ensuing step involved the creation of first-strand cDNAs, employing random hexamer primers and Reverse Transcriptase (RNase H), with mRNA fragments as templates. Following this, the process of second-strand cDNA synthesis took place using DNA polymerase I, RNAseH, buffer, and dNTPs. The resultant double-stranded cDNA fragments were then linked to adapters to construct the paired-end library. An additional step encompassed PCR amplification of the adaptor-ligated cDNA. The ensuing PCR product was refined through the AMPure XP system, and the library's quality was assessed using the Agilent Bioanalyzer 4150 system. Ultimately, the

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prepared library was subjected to sequencing on the Illumina Novaseq 6000 platform, generating paired-end reads with a length of 150 base pairs.

2.7 | Bioinformatics analysis

The chromatographic peak area and migration time were obtained for targeted metabolomics using the Multiquant 3.0.2 version. Correct the migration time using the standard of the target substance for metabolite identification. *T*-test was applied for a remarkable difference analysis between high-activity and low-activity groups. Fold change (FC) calculation was based on the average of metabolites in high-activity and low-activity groups, the FC > 1 indicated upregulated metabolites, whereas FC < 1 indicated downregulated metabolites. For proteomics, $\log_2$ FC was conducted using the average protein abundance ratio in high- and low-activity groups. The criteria for selecting remarkably differentially abundant proteins were $p < .05$ and the FC > 1.2 or FC < 0.83. The protein data were performed using of uniprot_Ovis_aries_49010_20221213.fasta (https://www.uniprot.org/uniprot). The kyoto Encyclopedia of Genes and GUnidentifiedenomes (KEGG) pathway analysis of the different abundance proteins and differential metaboloties was performed using the online database (http://geneontology.org/). Enrichment analysis employed Fisher's exact test, considering functional categories and pathways with a significance level of $p < .05$. Regarding transcriptomics, FeatureCounts (http://subread.sourceforge.net/) was applied to quantify the reads aligned to each gene. Subsequently, the fragments per kilobase million for each gene were computed according to its length and the corresponding read count. Differential expression analysis was performed using DESeq2 (http://bioconductor.org/packages/release/bioc/html/DESeq2.html). Genes displaying $|\log_2 FC| > 2$ and $p_{adj} < .05$ were identified as significantly differentially expressed genes.

2.8 | Statistical analysis

Data analysis was conducted by SPSS Statistics 26.0 (IBM Corp). A *T*-test was performed to analyze remarkable differences in the activity of PGK1 and PKM2, meat quality traits, and differential metabolites among different activity groups. The GraphPad Prism 9.3 software, Origin (version 2022b), and R version 4.3.1 were utilized to produce visual patterns. All data are presented as the mean ± standard error.

3 | RESULTS

3.1 | Meat quality traits of the postmortem muscle with different PGK1 and PKM2 activity

Our previous studies discovered a close association between PGK1 and PKM2 and postmortem meat quality traits, and they could be considered promising biological indicators for characterizing meat quality (Huang, Blecker, et al., 2023; Huang, Zhang, et al., 2023). In order to clarify the biological mechanism through which PGK1 and PKM2 regulate postmortem meat quality. We measured the PGK1 and PKM2 activity changes in the LT muscle of 60 lamb carcasses at 24 h postmortem. The two groups (high-activity and low-activity groups, $n = 10$) were selected based on the different activity levels of PGK1 and PKM2 activity. As presented in Figure 1a,b, the activity of PGK1 and PKM2 in high-activity group was remarkably greater than those in low-activity ($p < .001$). Moreover, the meat quality assessment results observed that in high-activity group, shear force (Figure 1c) and cooking loss (Figure 1d) were noticeably lower compared with low-activity group ($p < .01$). Conversely, the a^* (Figure 1e) and R630/580 values (Figure 1f) were noticeably greater in high-activity group than low-activity group ($p < .05$). It was suggested that the activity of PGK1 and PKM2 was closely associated with the postmortem meat quality traits. These findings are consistent with our previous results (Huang Zhang et al., 2023).

3.2 | Targeted metabolomics analysis of the postmortem muscle with different PGK1 and PKM2 activities

Energy metabolism is one of the crucial biochemical pathways that determine meat quality during the transformation of muscle to meat. The primary processes in postmortem energy metabolism are the depletion of ATP and the accumulation of H^+ (Hamm, 1977). In postmortem muscle, the critical biological pathways involved in energy metabolism mainly include glycolysis, oxidative phosphorylation, and the tricarboxylic acid (TCA) cycle. Therefore, in order to elucidate the impact of PGK1 and PKM2 on postmortem muscle at the metabolome level, the targeted metabolomics—MRM energy metabolism approach was used to identify the metabolites involved in energy metabolism processes. A total of 26 metabolites were successfully quantified in different activity groups (high- and low-activity) using the MRM-targeted metabolomics approach (Figure 2). Among these 26 quantified metabolites, 13 metabolites showed the differential expression based on the standards of $p < .05$ and FC > 1 for upregulated metabolites and FC < 1 for downregulated metabolites. These metabolites included glucose-6-phosphate, nicotinamide adenine dinucleotide phosphate (NADP), fructose-6-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, pyruvate, and lactate in the glycolytic pathway, as well as citrate, fumarate, and isocitrate involved in the TCA cycle pathway. Additionally, adenosine diphosphate (ADP), ATP, and nicotinamide adenine dinucleotide (NAD^+) simultaneously participated in glycolysis, TCA cycle, and oxidative phosphorylation pathways (Figure 3). Subsequently, in order to display the differences in the expression patterns of metabolites across different activity groups in a more comprehensive and intuitive manner, the content of 13 key metabolites was calculated using a distance matrix, and hierarchical clustering was applied for the clustering analysis (Figure S1A). The intensity of red color corresponded to higher expression levels of metabolites, whereas the intensity of blue color corresponded to lower

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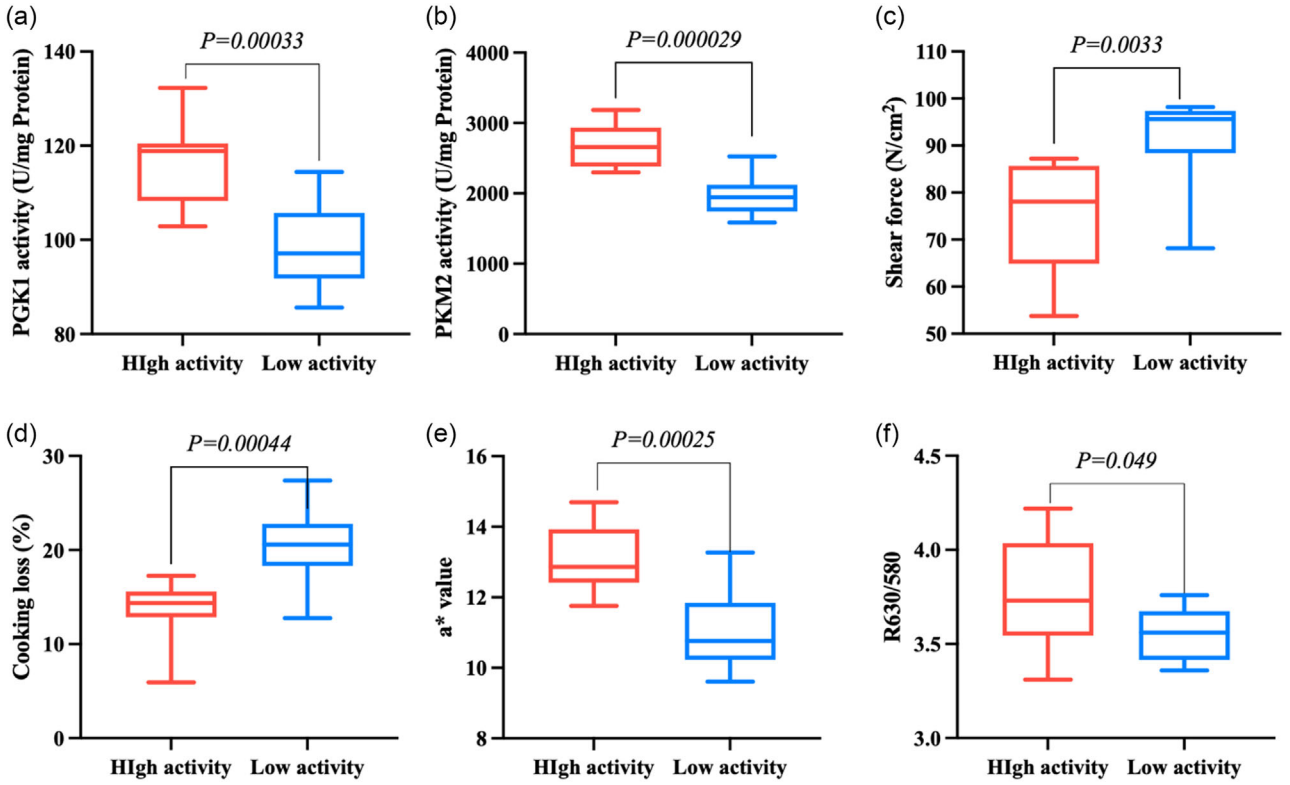

FIGURE 1 Selection of the postmortem muscle samples with the different activity of phosphoglycerate kinase 1 (PGK1) (a) and pyruvate kinase M2 (PKM2) (b), and their difference in meat quality traits: tenderness (c), water holding-capacity (d), and color (e and f).

expression levels of metabolites. The results revealed that 13 different metabolites exhibited similar metabolic patterns. Moreover, principal component analysis found that 13 differential metabolites could significantly distinguish the 2 different activity groups (Figure S1B). In order to clarify the relationship between the 13 critical differential metabolites and the activity of PGK1 and PKM2, correlation analysis results observed that the activity of PGK1 and PKM2 showed a positive correlation with NADP, citrate, fumarate, isocitrate, ADP, and NAD^+, whereas negatively correlated with glucose-6-phosphate, fructose-6-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, pyruvate, lactate, and ATP (Figure S1C). Therefore, it was indicated that glucose-6-phosphate, NADP, fructose-6-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, pyruvate, lactate, citrate, fumarate, isocitrate, ADP, ATP, and NAD^+ may be considered the key metabolites that were influenced and regulated by the activity of PGK1 and PKM2.

3.3 | Proteome analysis of the postmortem muscle with different PGK1 and PKM2 activity

We subsequently proceeded to delineate the proteome profile associated with the different activity of PGK1 and PKM2 using of the TMT-quantitative proteomics approach. The findings indicated that a total of 2355 proteins were identified, of which 2352 proteins were quantified in both high- and low-activity groups (Figure S2A). Among these 2352 quantifiable proteins, the differential abundance proteins were screened based on the criteria of $p < .05$ and $\log_2$ FC > $\log_2(1.2)$ as upregulated proteins and $\log_2$ FC < $\log_2(0.83)$ as downregulated proteins. A total of 90 differential abundance proteins were identified in high- and low-activity groups, including 52 upregulated differential proteins and 38 downregulated differential proteins (Figure S2B). In order to demonstrate the dramatic differences in protein expression in high- and low-activity groups, a volcano plot was drawn using the $\log_2$ FC and *p*-value as two parameters (Figure S2C). Proteins exhibiting significant downregulation were annotated in blue, whereas those significantly upregulated were denoted in red. Proteins with no noteworthy expression changes were depicted in gray. Figure S2D presents the heatmap of differentially abundant proteins in high- and low-activity groups. Significant differences can be observed between different groups. To visualize the key pathways and mechanisms that account for the differential abundance proteins, we compiled all the differential abundance proteins, as annotated by KEGG, to further investigate their functions within different activity groups. It was observed that these proteins were predominantly involved in 25 pathways (Figure S2E). These included cysteine and methionine metabolism, metabolic pathways, propanoate metabolism, glycolysis, muscle contraction, ECM-receptor interaction, HIF-1 signaling pathway, carbon metabolism, apelin signaling pathway, pentose phosphate pathway, TCA cycle, fructose and mannose metabolism, alanine, aspartate and glutamate metabolism, pyruvate metabolism, glutathione metabolism, mitophagy-animal, PI3K-Akt signaling pathway, biosynthesis of amino acids, peroxisome, PPAR signaling

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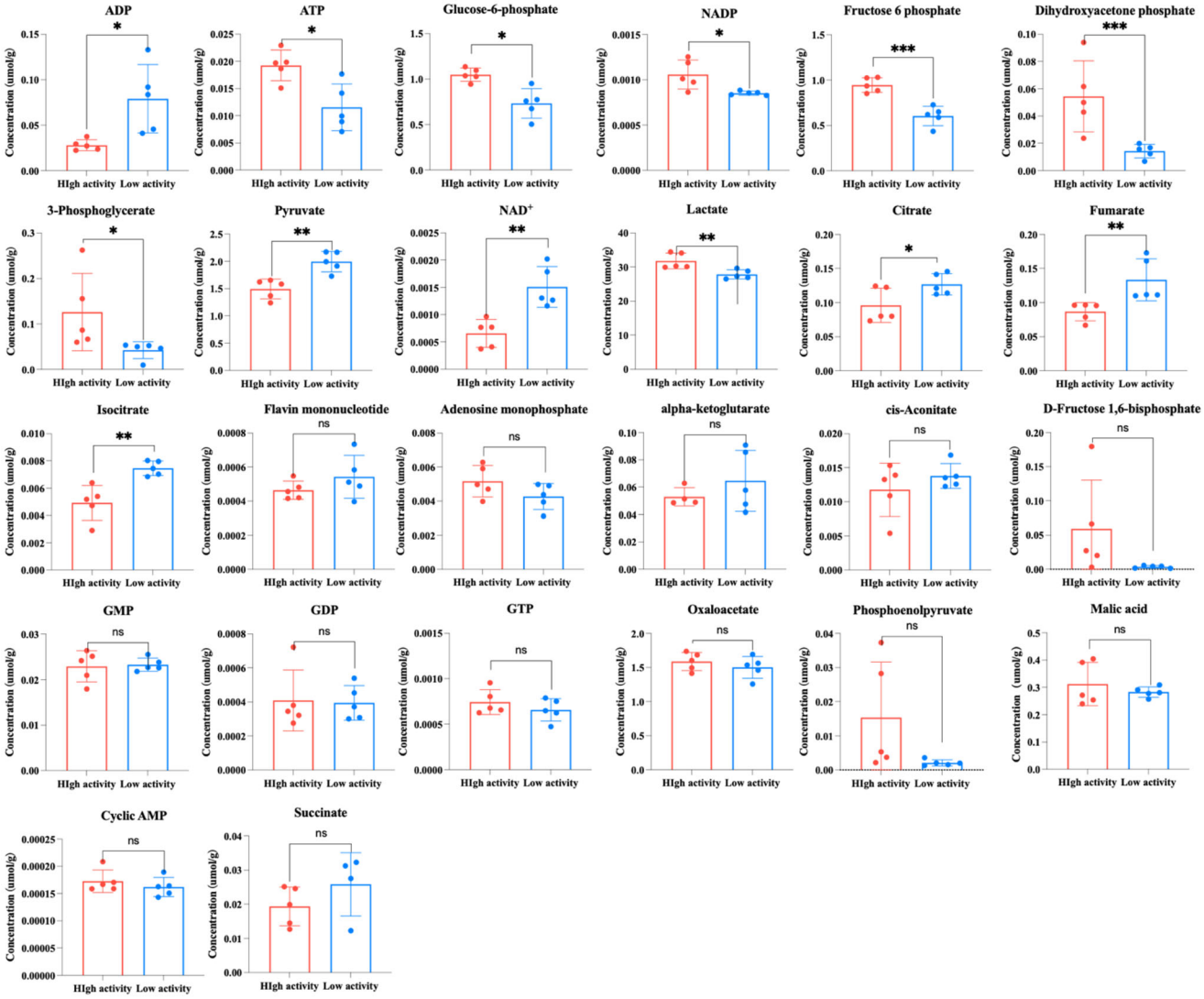

FIGURE 2 Quantitative analysis of energy metabolites was conducted using a targeted metabolomics approach based on multiple reaction monitoring (MRM).

pathway, lysosome, oxidative phosphorylation, cellular senescence, RNA transport, and regulation of actin cytoskeleton. In KEGG pathway enrichment analysis, it has been determined that the following 17 proteins—EIF3C, AP4E1, FABP3, SQSTM1, COL1A2, TNXB, MYL3, MYL2, fructose bisphosphate aldolase B (ALDOB), DDO, SUCLG1, GSTM3, CA3, NDUFB8, MRI1, lactate dehydrogenase B (LDHB), and BHMT2—participated in these biological pathways (Figure 4). This suggested that the activity of PGK1 and PKM2 could regulate these different abundance proteins and their biological pathways, thereby affecting the development of postmortem meat quality.

3.4 | Transcriptome analysis of the postmortem muscle with different PGK1 and PKM2 activity

In addition, we also evaluated the influence of PGK1 and PKM2 activity on muscle transcriptome through RNA-seq analysis of lamb muscle samples from both the high- and low-activity groups. The results indicated that a total of 312 differentially expressed genes at padj < 0.05 and $|\log_2(FC)| > 2$: 123 upregulated and 189 downregulated transcripts in different activity groups (Figure S3A). The results of clustering analysis for differentially expressed genes are presented in Figure S3B. The clustering heatmaps used red and blue colors to represent the high or low gene expression, respectively. The KEGG pathway enrichment analysis revealed that the differentially expressed genes in high- and low-activity groups were primarily enriched in pathways related to carbon metabolism, purine metabolism, metabolic pathways, fructose and mannose metabolism, glycine, serine and threonine metabolism, cysteine and methionine metabolism, glycolipid metabolism, apoptosis, glycolysis, peroxisome, muscle contraction, pantothenate and CoA biosynthesis, ECM-receptor interaction, glycerophospholipid metabolism, pentose phosphate pathway, galactose metabolism, starch and sucrose metabolism, biosynthesis of unsaturated fatty

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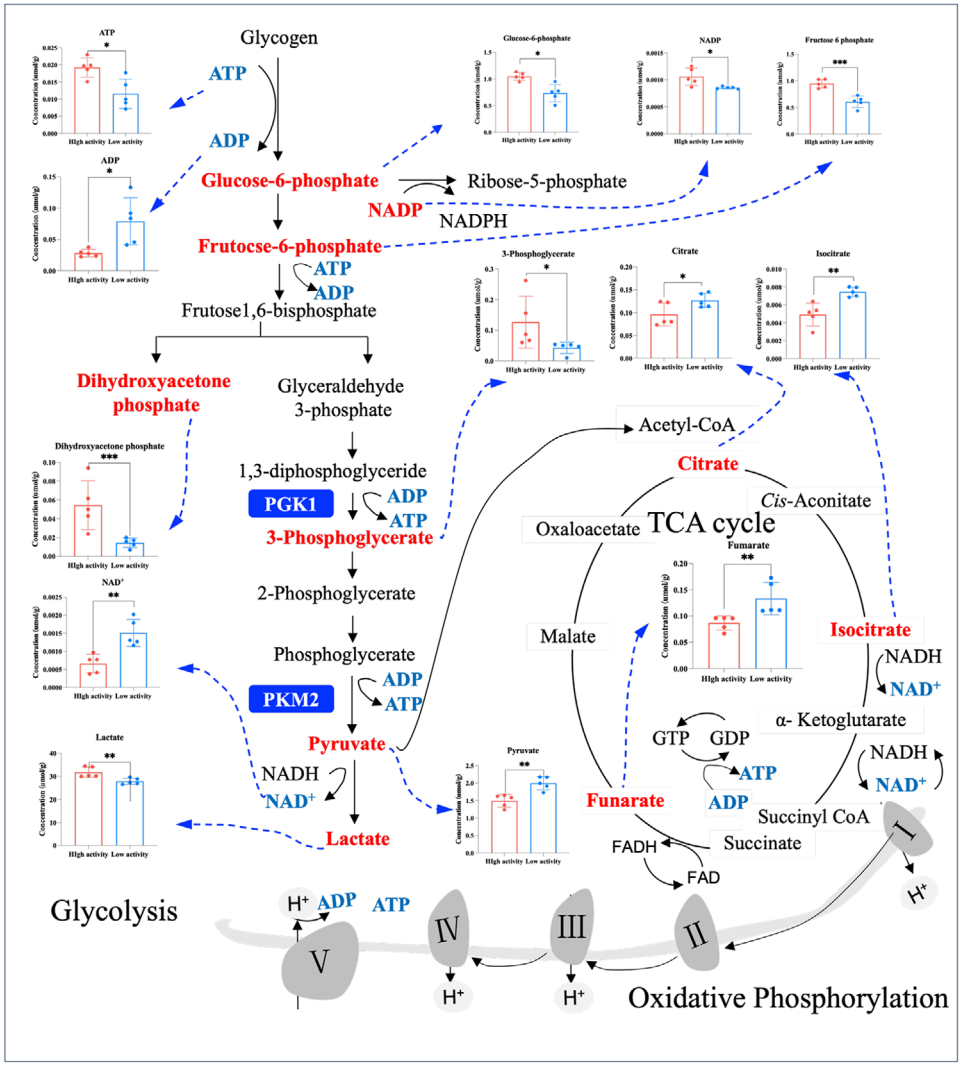

FIGURE 3 The schematic diagrams of 13 key metabolites related to phosphoglycerate kinase 1 (PGK1) and pyruvate kinase M2 (PKM2) activity involving in energy metabolism pathways.

acids, proteasome, endocrine and other factor-regulated calcium reabsorption, antifolate resistance, MAPK signaling pathway, glutathione metabolism, calcium signaling pathway, regulation of actin cytoskeleton, PPAR signaling pathway, PI3K-Akt signaling pathway, HIF-1 signaling pathway, AMPK signaling pathway, lysosome, cell cycle, apelin signaling pathway, ubiquitin mediated proteolysis, and RNA transport (Figure S3C). Moreover, there were 32 differentially expressed genes that were closely associated with these biological pathways (Figure 5), including UBE2L6, BUB1, SORBS1, CYFIP2, PDGFRB, MAP4K1, PSMB8, COL6A6, THBS1, VNN1, TNNT2, CACNA2D3, BCL2A1, APAF1, CTSS, ELOVL7, TH, GPX2, PMVK, GPAT3, LPIN1, TYMS, SGPL1, PLCB1, XDH, PDE7B, AMPD3, GUCY1A1, PHGDH, fructose-1,6-bisphosphatase 1 (FBP1), SDSL, and hexokinase (HK). It was suggested that the activity of PGK1 and PKM2 may affect and regulate the above-mentioned differential expression genes and their biological pathways.

3.5 | Integrated multi-omics for the different activity of PGK1 and PKM2 in postmortem muscle

To gain deeper insights into the molecular mechanisms underlying the impact of glycolytic enzymes (PGK1 and PKM2) on postmortem meat quality, we conducted a comprehensive integration and analysis of metabolomics, proteomics, and transcriptomics data to investigate the influence of PGK1 and PKM2 on meat quality across metabolic, protein, and RNA levels as well as elucidate their regulatory mechanisms. As presented in Figure S4A, we performed the Venn diagram analysis for the key biological pathways related to the different activity of PGK1 and PKM2 in the metabolomics, proteomics, and transcriptomics analysis. The results revealed that glycolysis was a critical biological pathway at the metabolome, proteome, and transcriptome levels. Therefore, we analyzed the differential metabolites, proteins, and genes involving in the glycolysis pathway at metabolome, proteome, and transcriptome levels. The results showed that ATP, ADP,

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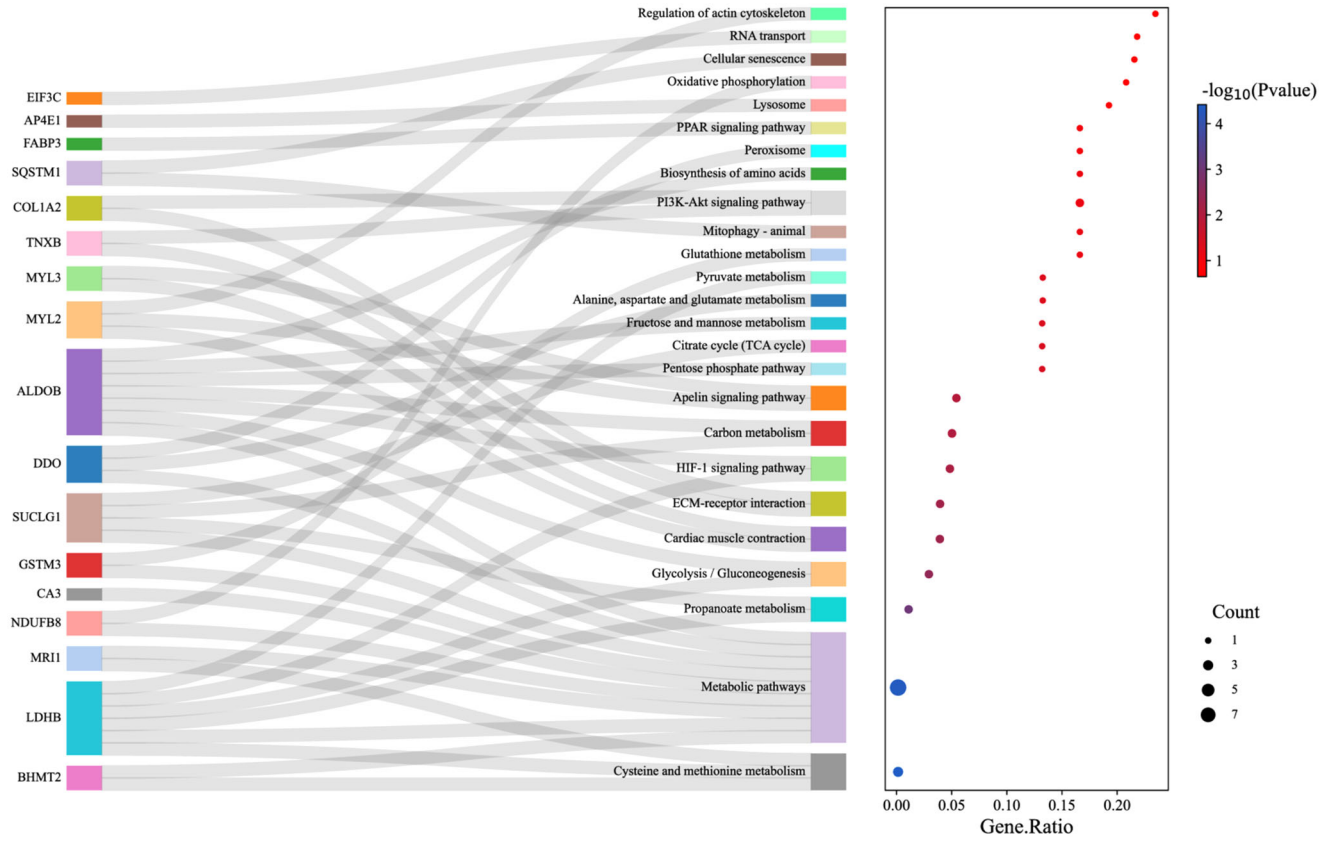

FIGURE 4 KEGG pathway enrichment analysis of differential abundance proteins in different phosphoglycerate kinase 1 (PGK1) and pyruvate kinase M2 (PKM2) activity groups using proteomic approach.

glucose-6-phosphate, NADP, fructose-6-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, NAD^+, lactate, and pyruvate were present in the metabolome, LDHB and ALDOB were present at the proteome level, as well as HK and FBP1 were present at the transcriptome level (Figure S4B). Additionally, we also found that TCA cycle and oxidation phosphorylation were also the common biological pathways in metabolome and proteome levels (Figure S4A). Moreover, there were a total of 16 biological pathways involved in both the proteome and transcriptome simultaneously (Figure S4A). Among then, muscle contraction has been considered the critical biological pathway to effect and regulate postmortem meat quality. Thus, we summarized the roles of these differential substances in the glycolytic pathway and their relationship to the TCA cycle, oxidation phosphorylation, and muscle contraction as shown in Figure 6. We inferred that the activity of PGK1 and PKM2 could regulate and affect glycolytic process and then impact the postmortem meat quality. Moreover, the TCA cycle, oxidation phosphorylation, and muscle contraction could also be affected by the activity of PGK1 and PKM2.

4 | DISCUSSION

The tenderness, color, and water-holding capacity of fresh meat significantly influence and determine the consumers' purchase intention and the economic benefits of the meat industry. Furthermore, the deterioration of meat quality, characterized by issues such as discoloration, significant juice loss, and deterioration in the texture of postmortem muscle, can contribute to meat resource waste and environmental pollution. Thus, the production of high-quality meat is particularly important in industry. The transformation of postmortem muscle into meat involves a series of intricate physiological and biochemical changes, ultimately determining the development of meat quality (Matarneh et al., 2021). PGK1 and PKM2 are the two ATP-generating enzymes in glycolysis, and they play a crucial role in controlling the glycolytic rate, thereby impacting and determining meat quality (Hamm, 1977; Honikel, 2014). Previous studies have demonstrated that glycolytic enzymes (PGK1 and PKM2) can be identified as the protein biomarkers to assess the postmortem meat quality (Huang, Blecker, et al., 2023; Huang, Zhang, et al., 2023; Kim et al., 2019; Ren et al., 2022). In this work, we divided the lamb muscle samples into high-activity and low-activity groups according to PGK1 and PKM2 activity at 24 h postmortem. The findings indicated that the high-activity group exhibited elevated a^* and R630/580 values, as well as reduced shear force and cooking loss compared to the low-activity group. This agreed with our previous study (Huang, Zhang, et al., 2023). Therefore, we conducted the targeted metabolomics, proteomics, and transcriptomics analysis on both the high- and low-activity groups to reveal the molecular mech-

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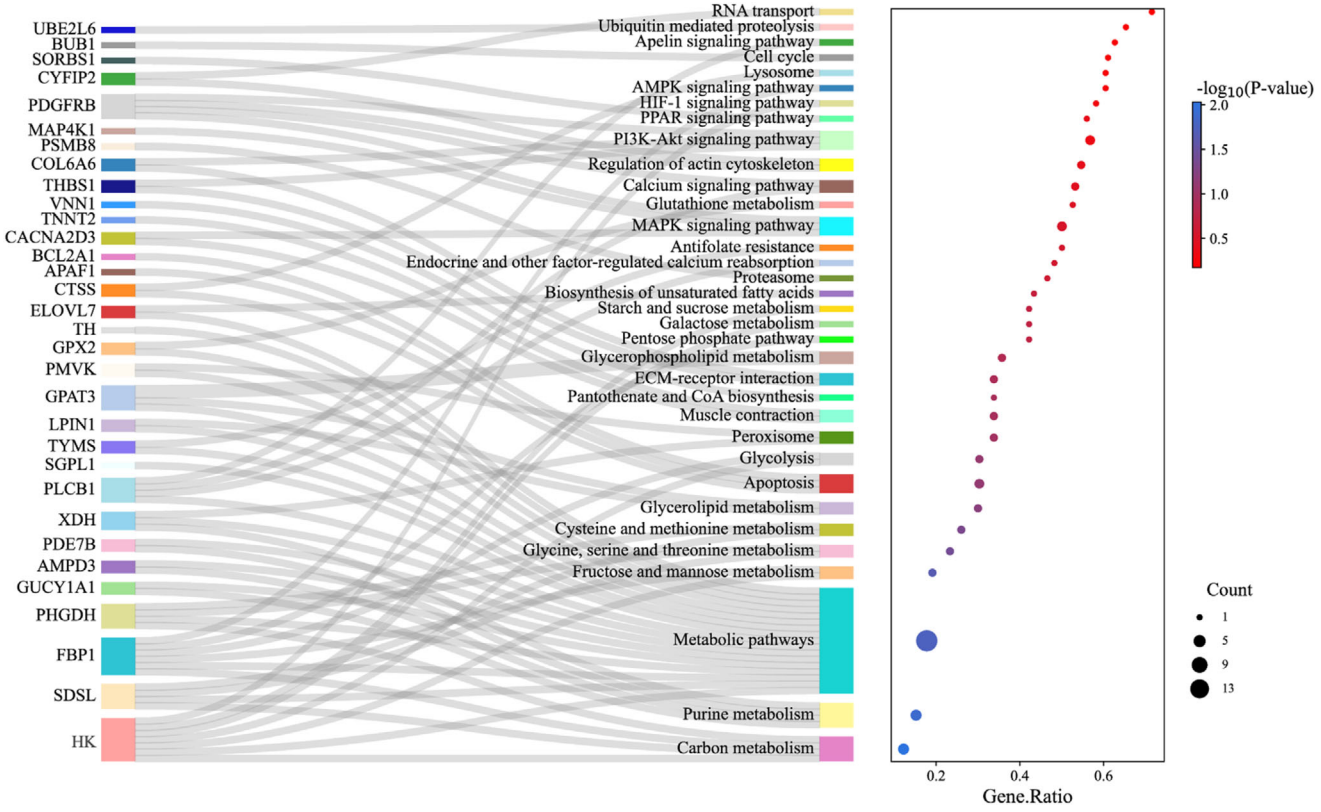

FIGURE 5 KEGG pathway enrichment analysis of the differentially expressed genes in different phosphoglycerate kinase 1 (PGK1) and pyruvate kinase M2 (PKM2) activity groups using transcriptomics approach.

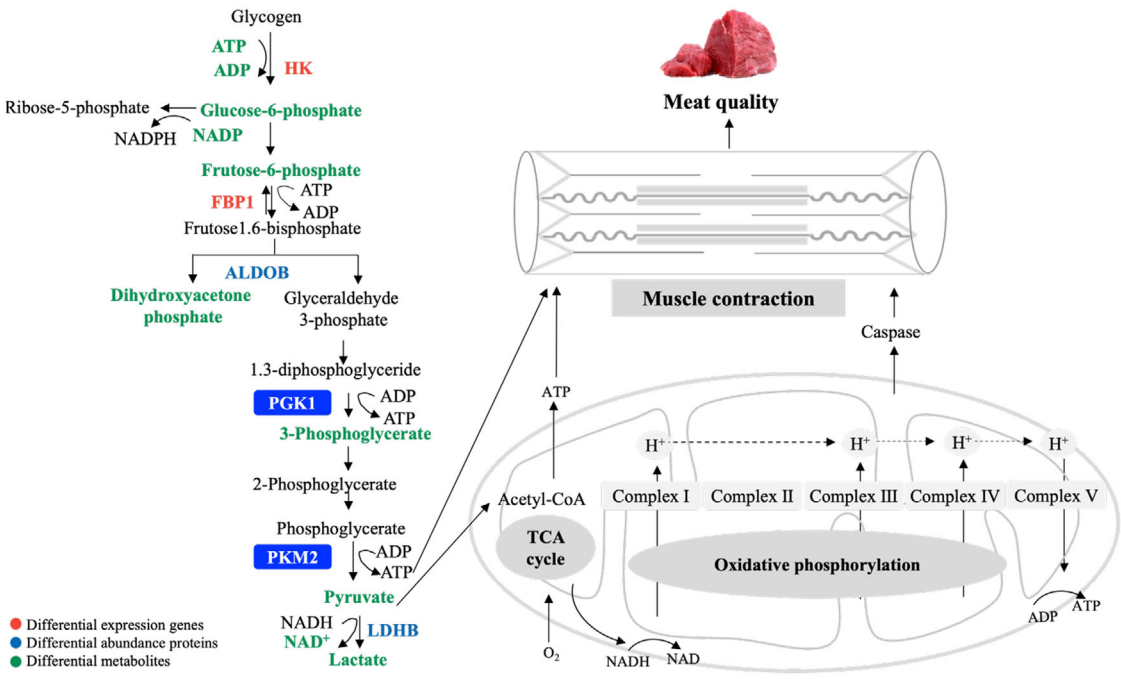

FIGURE 6 Summary of the critical differential genes, proteins, and metabolites involved in glycolysis and their relationship with the tricarboxylic acid cycle (TCA cycle), oxidative phosphorylation, and muscle contraction.

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anisms by which PGK1 and PKM2 characterize the postmortem meat quality.

In present study, a total of 13 differential metabolites, 90 differential abundance proteins, and 312 differentially expressed genes ($p < 0.05$) were identified in different activity of PGK1 and PKM2 groups by targeted metabolomics, proteomics, and transcriptomics analysis, respectively. Results showed that glycolysis was a crucial biological pathway at the metabolome, proteome, and transcriptome level. Glycolysis, an energy-metabolism process in postmortem muscle, holds significant importance in influencing the progression of postmortem meat quality. Generally, excessive glycolysis can lead to the formation of PSE (pale, soft, and exudative) meat or meat with characteristics resembling PSE, whereas insufficient glycolysis can result in the formation of DFD (dark, firm, and dry) meat (Briskey, 1964). Moreover, the previous studies also reported that glycolysis was closely related to meat quality by omics approaches. For instance, a study conducted by Lu et al. (2023) demonstrated that glycolysis was a notable metabolic pathway influenced by protein *S*-nitrosylation, which played a role in regulating postmortem muscle energy metabolism. Yu et al. (2019) found that energy metabolites in bovine muscle during the early postmortem period were determined by the glycolytic pathway using of targeted metabolomics. For the proteome level, numerous studies also reported that the postmortem meat quality traits were influenced and regulated by the glycolytic pathway (Huang, Zhanget al., 2023; Huang et al., 2020; Setyabrata et al., 2023; Xu et al., 2023). Additionally, a prior investigation identified that glycolysis-related enzyme activities played a pivotal role in the formation of PSE pork, as determined through transcriptome-based analysis (Zequan et al., 2022). In a study conducted by Zhan et al. (2022), an integrated analysis of transcriptomic and metabolomic profiles was performed, revealing the complex molecular regulatory network that underlies meat quality. The findings highlighted that the differentially expressed genes related to intramuscular fat (IMF) of pork were notably enriched in glycolysis. Thus, glycolysis could be considered a key biological pathway that was regulated by the activity of PGK1 and PKM2, thereby affecting the postmortem meat quality.

The results observed the presence of ATP, ADP, glucose-6-phosphate, NADP, fructose-6-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, NAD^+, lactate and pyruvate at the metabolome level. Additionally, LDHB and ALDOB were detected at the proteome level, whereas HK and FBP1 were identified at the transcriptome level. It was suggested that the activity of PGK1 and PKM2 could regulate these differential metabolites, proteins, and genes. Metabolomics aims to identify small-molecule metabolites (<1500 Da) within an organism, using modern detection techniques to comprehensively determine and analyze the diverse array of metabolites present in the biological system (Creydt & Fischer, 2018). In a study performed by Ma et al. (2023), they found that meat quality between two local breeds of Tibetan sheep was affected by the differential metabolites (glucose-6-phosphate, fructose-6-phosphate) through using metabolomics approach. Lu et al. (2023) found that the differential energy metabolites (glucose-6-phosphate, pyruvate, and lactate) were affected by protein *S*-nitrosylation at early postmortem pork. Moreover, the findings observed that the energy metabolites (glucose-6-phosphate, fructose-6-phosphate, dihydroxyacetone phosphate, pyruvate, lactate, ATP, ADP, NADP, and NAD^+) exhibited the significant difference in *Longissimus lumborum* and *Psoas Major* muscles during the early stages of postmortem aging (Yu et al., 2019). Cai et al. (2019) found that enhancing PGK1 activity could increase glycolysis by increasing cellular ATP levels. In this work, the differential metabolites (ATP, ADP, glucose-6-phosphate, NADP, fructose-6-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, NAD^+, lactate, and pyruvate), as important metabolites in glycolysis, were significantly different in two PGK1 and PKM2 activity groups. This result demonstrated that PGK1 and PKM2 activity could regulate the rate of glycolysis by influencing glycolytic metabolites. The previous findings suggested that accelerating the postmortem glycolytic rate contributes to the ATP production and glycogen degradation, which, in turn, affects the pH decline rate and subsequently influences the meat quality characteristics (Chen et al., 2022). Consistent with our results, Bai et al. (2020) revealed that the content of glycogen, lactate, glucose, and glucose-6-phosphate in postmortem lamb muscle could influence the glycolytic rate. This may be due to that PKM2, a glycolytic rate-limiting enzyme, reportedly plays an important role in energy metabolism.

At proteome level, the findings observed an upregulation of differential abundance proteins (LDHB and ALDOB) in the high-activity group and a downregulation in the low-activity group. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate in the last step of glycolysis. It is composed of two subunits, encoded by the LDHA and LDHB, respectively (Liu et al., 2023). In glycolysis, ALDOB catalyzes the transformation of fructose 1,6-diphosphate to dihydroxyacetone phosphate, and glyceraldehyde-3-phosphate, which is an indicator of glycolytic activity in postmortem (Reiche et al., 2019). LDHB and ALDOB are also two critical glycolytic enzymes in glycolysis pathway. The previous studies found that they were closely associated with postmortem meat quality. For instance, Picard et al. (2015) found that LDHB was negatively correlated with meat tenderness by proteomics analysis. Santiago et al. (2023) reported that steers with higher levels of ALDOB abundance, carcass fatness, and meat color, as well as lower ultimate pH and shear force, in comparison to bulls. This was in accordance with our results. Moreover, ALDOB plays a role in various cellular functions and biological processes associated with muscle maintenance, regulation of cell shape and mobility, striated muscle contraction, actin filament organization, and ATP biosynthetic processes (Ji et al., 2016). López-Pedrouso et al. (2023) also observed a strong association between LDHB and IMF in foal meat, whereas ALDOA displayed significant correlations with shear force and color of foal meat, as determined through the proteomics approach. Moreover, a study performed by Chen, L., Li, Z., Everaert, N., Lametsch, R., & zhang, D. (2019), they observed the phosphorylation level of fructose-bisphosphate aldolase with a lower level in fast glycolytic rate group.

Moreover, the differentially expressed genes (HK and FBP1) were also identified within the glycolysis pathway in response to the different activities of PGK1 and PKM2 at transcriptome level. HK functions

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as a key rate-limiting enzyme in glycolysis and plays a crucial role in catalyzing the transformation of glucose to glucose-6-phosphate within postmortem glycolysis (Li et al., 2016). Zequan et al. (2021) reported that the activity of HK in PSE meat was higher than that in normal meat at 24 h postmortem. The findings reported that preslaughter stress can enhance HK activity in early postmortem, thus affecting meat quality (Li et al., 2016). FBP1 is the rate-limiting enzyme in gluconeogenesis, which is crucial in catalyzing enzymatic reactions (Zhang et al., 2021). López-Pedrouso et al. (2023) revealed a strong connection between FBP1 and IMF, but no significant correlation was found between FBP1 and shear force in foal meat. A study also conducted by Chen, D., Li, W., Du, M., & Cao, B. (2019), they observed that FBP1 had a higher level in the low marble of beef cattle than that in the high marble group. Overall, it was indicated that differential metabolites (ATP, ADP, glucose-6-phosphate, NADP, fructose-6-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, NAD^+, lactate, and pyruvate), differential abundance proteins (LDHB and ALDOB), and differentially expressed genes (HK and FBP1) within the glycolysis could influence the glycolytic rate, thereby influencing the formation of postmortem meat quality. This is due to that the glycolysis is an energy-metabolism process in postmortem muscle, which plays an important role in deciding the postmortem meat quality (Briskey, 1964). Additionally, PGK1 and PKM2 are the two ATP-generating enzymes in glycolysis, and they play a crucial role in controlling the glycolytic rate (Hamm, 1977; Honikel, 2014). Therefore, it was suggested that PGK1 and PKM2 may regulate these key substrates in the glycolytic pathway at different molecular levels to affect and decide the formation of postmortem meat quality (Figure 6).

In postmortem skeletal muscles, muscle contraction refers to the establishment of enduring tension, characterized by the swift intertwining of thin and thick filaments within the sarcomeres to a notable extent. This occurs when ATP is depleted and Ca^{2+} is released from the sarcoplasmic reticulum (Muroya et al., 2007). In present study, we found that muscle contraction was significantly enriched at both proteome and transcriptome levels. In addition, the KEGG pathway analysis also revealed significant enrichment of the TCA cycle and oxidative phosphorylation pathways at both the proteome and metabolome levels. The TCA cycle is a significant pathway for muscle energy and substance metabolism in postmortem muscle, leading to energy generation (Jia et al., 2006). Oxidative phosphorylation is the coupled reaction involving ADP and inorganic phosphate to generate ATP. This process is responsible for generating the majority of ATP in organisms (Nolfi-Donegan et al., 2020). ATP functions as the cellular energy unit. The energy discharged through the hydrolysis of ATP's high-energy phosphate bond can be harnessed for mechanical functions like muscle contraction, chemical activities encompassing biosynthesis and assimilation, and active transport. The consumption of ATP is counterpoised by energy-generating routes that dismantle reserved nutrients (such as glycogen or fats) to synthesize ATP (Wang et al., 2022). Therefore, it was suggested that PGK1 and PKM2 are two ATP-generating enzymes involved in the glycolytic pathway, which may affect energy metabolism and then influence muscle contraction, TCA cycle, and oxidation phosphorylation pathways (Figure 6).

5 | CONCLUSION

This study investigated the molecular mechanism of biomarkers (PGK1 and PKM2) characterizing the postmortem meat quality using metabolomics, proteomics, and transcriptomics techniques. The results observed that 13 key differential metabolites related to PGK1 and PKM2 activity were identified using the targeted metabolomics, mainly involved in glycolysis, TCA cycle, and oxidative phosphorylation. At the proteome level, a total of 90 differential abundance proteins were identified in high- and low-activity groups by TMT-proteomics, which were mainly involved in the biological pathways of glycolysis, muscle contraction, TCA cycle, and so on. RNA-seq results observed that 312 differentially expressed genes were identified in different activities of PGK1 and PKM2 muscle samples, and they were primarily enriched in pathways related to metabolic pathways, glycolysis, muscle contraction, and so on. Moreover, multi-omics integration analysis revealed that glycolysis emerges as a pivotal biological pathway exerting a collective influence on the activity of PGK1 and PKM2 across metabolomic, proteomic, and transcriptomic levels. Notably, the differential metabolites (ATP, ADP, glucose 6-phosphate, NADP, fructose-6-phosphate, dihydroxyacetone phosphate, 3-phosphate, NAD^+, lactate, and pyruvate), differential abundance proteins (LDHB and ALDOB), and differentially expressed genes (HK and FBP1) were closely associated with the regulation of PGK1 and PKM2 activity. It was concluded that PGK1 and PKM2 may affect and regulate the different molecular level metabolites, proteins, and genes in glycolysis pathway, thereby impacting the changes in postmortem meat quality. In addition, PGK1 and PKM2 could regulate the energy metabolism to influence postmortem muscle contraction, TCA cycle, and oxidation phosphorylation pathways. This study comprehensively utilized transcriptome, proteome, and metabolomic analysis methods to reveal the molecular mechanisms of PGK1 and PKM2 characterizing the postmortem meat quality. These findings provide new perspectives and potential targets for regulating the postmortem meat quality.

AUTHOR CONTRIBUTIONS

Caiyan Huang: Conceptualization; methodology; software; writing—original draft; writing—review and editing. **Can Xiang**: Writing—review and editing. **Fangzhou Wang**: Writing—review and editing. **Christophe Blecker**: Supervision. **Zhenyu Wang**: Investigation; supervision. **Li Chen**: Conceptualization; investigation; validation; supervision. **Dequan Zhang**: Funding acquisition; project administration; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known financial conflicts of interest or personal relationships that could have appeared to influence the work reported in this paper.

ETHICS STATEMENT

This study was approved by the Ethics Committee of the Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences (approval number: ISFT2022-006).

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SUPPORTING INFORMATION

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