Bioscience Methods 2025, Vol.16, No.2, 83-99
http://bioscipublisher.com/index.php/bm

Feature Review Open Access

Single-Cell Multi-Omics Analysis of Epigenetic and Transcriptional Regulatory Mechanisms of Goat Skeletal Muscle Development

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Bioscience Methods, 2025, Vol.16, No.2 doi: 10.5376/bm.2025.16.0009
Received: 21 Feb., 2025
Accepted: 31 Mar., 2025
Published: 11 Apr., 2025
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Preferred citation for this article:
Zhang X.Z., and Lin X.F., 2025, Single-cell multi-omics analysis of epigenetic and transcriptional regulatory mechanisms of goat skeletal muscle development, Bioscience Methods, 16(2): 83-99 (doi: 10.5376/bm.2025.16.0009)

Abstract The normal development of goat skeletal muscle is crucial for the growth performance and meat quality of meat goats. This study focuses on the application of single-cell multi-omics technology in the study of goat skeletal muscle development and explores the key mechanisms of epigenetic and transcriptional regulation. This study first outlines the stages, cell types and molecular regulators of goat skeletal muscle development, emphasizing its species-specific characteristics. It then discusses how single-cell transcriptomics can identify cell subpopulations and gene expression profiles in muscle tissue, revealing the heterogeneity and differentiation trajectory of muscle lineage cells. The role of epigenetic mechanisms such as chromatin accessibility, histone modification and DNA methylation in muscle development is then explained, and the method of integrating transcriptome and epigenomic data to construct regulatory networks is introduced. Through case studies, the representative results of single-cell multi-omics in the study of livestock and poultry skeletal muscle development, such as the discovery of key signaling pathways and transcription factors, are summarized, and the implications of these findings for future research and genetic improvement are discussed. Finally, the application prospects of single-cell multi-omics in livestock research are prospected, and possible ways to translate research results into breeding practice are discussed, as well as the challenges faced at the technical, ethical and practical levels. This study aims to provide a comprehensive and systematic insight into the molecular mechanisms of goat skeletal muscle development and provide a scientific basis for genetic breeding and meat quality improvement of goats and other livestock.
Keywords Goat; Skeletal muscle development; Single-cell multi-omics; Transcriptional regulation; Epigenetic

1 Introduction

Goats are important livestock species, and the growth and development of their skeletal muscles directly affects the meat production and quality of meat goats (Deng et al., 2021; He and Li, 2024). Skeletal muscle is one of the most abundant tissues in mammals, accounting for about 40% of body weight (Xiong et al., 2022). Muscle development (myogenesis) is a complex and orderly process, which can generally be divided into the generation of muscle fibers in the embryonic period and the growth of muscle fibers after birth. During embryonic development, muscles originate from somitic cells in the mesoderm, undergo proliferation, differentiation and fusion of myogenic precursor cells to form protomyotubes, and further develop into primary and secondary muscle fibers (Cao et al., 2023). After birth, muscle fibers mainly achieve hypertrophy and growth through the proliferation and differentiation of satellite cells (i.e., muscle stem cells). Continuous muscle fiber hypertrophy will be limited if there is a lack of new nuclei contributed by satellite cells.

Therefore, muscle stem cells and their differentiation are crucial for muscle formation and growth, both in the embryonic period and in the growth stage. The muscle development process is strictly regulated by a variety of molecules such as the myogenic regulatory factors (MRFs) family. Among them, MyoD and Myf5 mainly determine the fate of myogenic precursors, and Myogenin and MRF4 (also known as Myf6) mediate the differentiation and fusion of myoblasts (Qiu et al., 2020; Zhou et al., 2022). The formation of muscle fiber types is regulated by specific transcription factors, such as NFATc1 and MEF2C, which contribute to the formation of slow muscle fibers, while FoxO1 promotes the formation of fast muscle fibers. Goat skeletal muscle development includes a series of precisely regulated stages in time and space, involving the coordination of multiple cell types and signal pathways.

Traditional transcriptomics is usually based on the average signal of tissues or cell populations, which makes it difficult to resolve the heterogeneity between different cell types. The rise of single-cell sequencing technology provides unprecedented resolution for in-depth analysis of complex tissues, and can characterize the transcriptional characteristics of cell types, states, and rare cell types at the single-cell level. In recent years, single-cell RNA sequencing (scRNA-seq) has been widely used in developmental biology and disease research, but its application in non-model animals such as livestock is relatively rare. In particular, in the field of muscle biology, single-cell transcriptomics can decompose skeletal muscle tissue into different subpopulations, revealing the transcriptional heterogeneity of muscle stem cells, myoblasts, and stromal cells, which is of great significance for understanding muscle development and regeneration (Qiu et al., 2017; Zhu et al., 2024). In addition, single-cell multi-omics is expected to construct a more comprehensive regulatory network by simultaneously or in parallel measuring multiple molecular levels (such as genome, transcriptome, and epigenome) of a single cell. For example, integrating single-cell transcriptome and epigenetic data can associate changes in gene expression with the dynamics of upstream regulatory elements (such as chromatin open regions or DNA methylation), thereby more directly revealing causal relationships (Ren et al., 2023). With the development of single-cell multi-omics technology and analytical methods, its application potential in muscle biology is huge, and it is expected to break through the limitations of traditional population-level research and provide new perspectives for analyzing the complex regulatory mechanisms of muscle development (Wang et al., 2020).

This study aims to systematically summarize the latest progress of single-cell multi-omics technology in the study of goat skeletal muscle development, focusing on the regulatory mechanisms at the transcriptional and epigenetic levels. This study first outlines the general process of goat skeletal muscle development, the main cellular and molecular players, and highlights the uniqueness of goats as a species in muscle development. Next, the insights of single-cell transcriptomics into goat muscle development are discussed, including the identification of different cell subpopulations, muscle lineage gene expression patterns, and important findings obtained from transcriptome data. Then, the epigenetic mechanisms related to muscle development are introduced, including the regulatory role of chromatin accessibility, histone modification, and DNA methylation in muscle cells, and how these mechanisms affect the expression of key muscle genes. After that, the application of multi-omics integrated analysis in discovering regulatory mechanisms was discussed, and the strategies for integrating transcriptome and epigenomic data, commonly used computational tools, and the advantages and limitations of multi-omics methods in muscle research were introduced. Then, by listing recent representative cases of single-cell multi-omics research on muscle development (including livestock muscle research), the understanding of epigenetic and transcriptional regulation in these studies, as well as the implications for future scientific research and livestock breeding practices, were summarized. Finally, the application prospects of single-cell multi-omics in livestock research were prospected, how to apply research results to genetic improvement was discussed, and challenges that need to be considered in terms of technical implementation and ethical practice were proposed. This study hopes to provide researchers with a comprehensive reference on the molecular regulation of goat skeletal muscle development, summarize the current understanding, and point out the direction for future work.

2 Overview of Goat Skeletal Muscle Development

2.1 Key stages of muscle generation

The process of goat skeletal muscle development can be divided into two main stages: embryonic muscle generation and postnatal muscle growth. During the embryonic period, the number of muscle fibers in adult skeletal muscle is basically determined: muscle stem cells originate from somites, undergo proliferation, migration and differentiation of myogenic precursor cells (myoblasts) during myometrial development, and primary myoblasts first differentiate and fuse to form primary myotubes, and then secondary myoblasts attach to primary myotubes and differentiate to form more secondary myotubes. These myotubes become muscle fibers when they mature. During myogenesis, a series of myogenesis regulatory factors play a core role, including Pax3/7, MyoD, Myf5, myosin heavy chain (MyHC), etc. Classic studies have shown that MyoD and Myf5 are essential factors for the formation of myogenic precursors, while Myogenin and MRF4 (Myf6) mediate the terminal differentiation of myoblasts (Qiu et al., 2020). For example, in early muscle progenitor cells and satellite stem cells, MyoD1 and

Myf6 are expressed at high levels, which helps promote their differentiation into myoblasts. After birth, the growth of muscle fibers is mainly achieved through the proliferation and differentiation of satellite cells. Satellite cells are adult stem cells located under the basement membrane of muscle fibers. They are usually in a quiescent state. Once they are damaged or stimulated by growth, they are activated to proliferate, and some new cells fuse into muscle fibers, thereby providing new nuclei to support muscle fiber hypertrophy. It should be noted that without satellite cells to supply new nuclei, the continuous hypertrophy of muscle fibers will be limited. Therefore, satellite cells play a key role in postnatal muscle growth and regeneration and repair. Throughout the process of muscle development, vascularization and innervation of muscle tissue also proceed synchronously to ensure that muscle fibers receive sufficient nutrition and signals. These steps together constitute the key stage of skeletal muscle development in goats and even mammals.

2.2 Cellular and molecular participants

The development of goat skeletal muscle involves the cooperation of multiple cell types and molecular participants. Muscle lineage cells include myogenic precursors, myoblasts and muscle fibers, as well as satellite cells that play an important role in the adult stage. In addition, there are non-myogenic stromal cells involved in regulating the microenvironment of muscle development, such as fibroblasts, adipocytes, and vascular endothelial cells. Among them, fibroblast/adipogenic progenitors (FAPs) are a type of mesenchymal stromal cells that have the potential to differentiate into fibroblasts or adipocytes and play a role in muscle damage repair and fat deposition. The latest study by Zhu et al. (2024) showed that there are multiple FAP subpopulations in goat skeletal muscle, and their differentiation trajectory is related to intramuscular fat (adipogenesis). Different cells in muscle tissue communicate through paracrine signals. For example, FAPs interact with satellite cells through growth differentiation factor (GDF), insulin-like growth factor (IGF) and other signals to affect muscle fiber formation and fat deposition.

At the molecular level, growth factors and cytokines constitute an important part of the muscle development regulatory network. For example, IGF-1 can promote myoblast differentiation and muscle fiber hypertrophy, while the TGF-β/Myostatin pathway inhibits myocyte proliferation and differentiation, thereby limiting muscle growth. Factors that regulate the conversion of muscle fiber types include calcium signaling pathways. The calcium-dependent protein phosphatase calcineurin can activate transcription factors such as NFATc1, which tends to favor the expression of slow muscle fiber genes. In contrast, the pathway activated by FoxO1 is conducive to the formation of fast muscle fiber characteristics (Figure 1) (Cao et al., 2023).

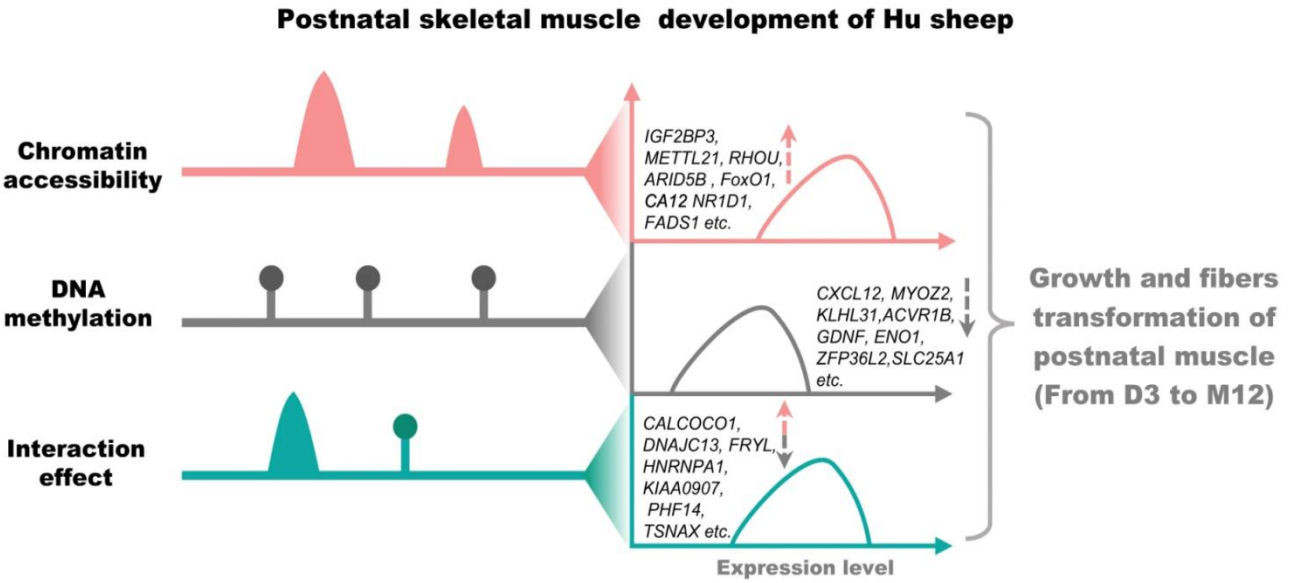

Figure 1 Summary of potential regulatory patterns of DNA 5mC and accessible chromatin regions on gene expression (Adopted from Cao et al., 2023)

Muscle-specific microRNAs are also important molecular participants. For example, miR-206 and miR-1 promote myogenic differentiation during myogenesis, while some miRNAs (such as the miR-29 family) affect fiber type and matrix remodeling (Xu et al., 2023). It is worth mentioning that long non-coding RNAs (lncRNAs) discovered in recent years also play a key role in muscle development. The study of Ye et al. (2023) showed that multiple lncRNAs regulate the expression of muscle genes through ceRNA mechanisms or by interacting with chromatin modification complexes. For example, specific lncRNAs were identified in the muscle development of cattle and sheep to affect muscle cell proliferation and differentiation. A study on bovine skeletal muscle by Zhang et al. (2020) found that lncRNA-403 regulates myogenesis by affecting the KRAS/Myf6 pathway; a study on small-tailed Hu sheep by Wu et al. (2020) showed that lncRNA CTTN-IT1 promoted satellite cell proliferation and differentiation by acting as a sponge for miR-29a to increase the expression of *YAP1*. The development of goat skeletal muscle is jointly participated by myogenic cells and stromal cells, and is precisely regulated by a series of signaling pathways, transcription factors, and non-coding RNA molecules.

2.3 Species-specific characteristics of goats

Although the basic mechanisms of skeletal muscle development are common among mammals, goats as a species also show some unique characteristics. First, the composition of muscle fiber types and fat deposition patterns in goat muscle may be different from those of other livestock species. For example, goat meat is generally considered to be leaner than beef and mutton, with lower intramuscular fat content, so the meat is characterized by low fat, low cholesterol, and high protein (Ren et al., 2023). This characteristic is related to both breed genetics and the differentiation behavior of adipocytes in its skeletal muscle. Recent single-nucleus transcriptome studies have shown that intramuscular adipogenesis in goat skeletal muscle plays an important role in muscle growth and significantly affects meat quality. Zhu et al. (2024) mapped the cell map of goat longissimus dorsi muscle development and found that different FAP subpopulations showed different transcriptional characteristics in the early and late stages of intramuscular adipogenic differentiation, and identified the transcription factor *TCF7L2* as a key regulatory factor in early adipogenesis. This finding suggests that the regulation of fat deposition in goat muscle may be species-specific and have a unique effect on goat meat quality.

Secondly, the diversity of goat breeds also brings about differences in muscle development characteristics. After long-term domestication and breeding, goats have formed many breeds with different economic traits. Some breeds grow rapidly and have large muscle mass, while others have unique muscle flavor or produce mainly cashmere. For example, Haimen goats in my country are small and grow slowly, but have outstanding cashmere production performance (Deng et al., 2021); while South African Boer goats grow fast and have high muscle yield. These differences between breeds are reflected in skeletal muscle development, including the rate of myofiber formation, the ratio of fiber types, and the content of intramuscular fat. Therefore, species and breed specificity need to be considered when studying goat skeletal muscle development. For example, a study on Guizhou white goats compared the longissimus dorsi muscles of individuals of different weights through transcriptome sequencing, and identified differentially expressed genes closely related to muscle growth and development, such as FGF11, NOTCH1, and ADIPOQ, which were significantly upregulated in the high-weight group (An et al., 2022). These genes are involved in cell proliferation and differentiation, fat metabolism, and growth factor signaling, and may have different expression patterns in goats of different breeds or growth types.

3 Insights into Goat Muscle Development from Single-Cell Transcriptomics

3.1 Identification of cell subpopulations

Single-cell RNA sequencing enables researchers to decompose complex muscle tissue into different cell subpopulations and to finely characterize the gene expression characteristics of each cell type. In goat skeletal muscle, the application of single-cell or single-nucleus transcriptome technology can identify a variety of cell types including satellite cells, myoblasts, myofibers, FAPs, immune cells, and endothelial cells. For example, Zhu et al. (2024) used single-nucleus RNA sequencing to analyze the longissimus dorsi muscle of goats at different developmental stages and constructed a detailed cell map. The results identified major cell populations such as myogenic satellite cells (MuSCs), different subtypes of fibroblasts/adipocyte precursors (FAPs), and other stromal cells. These subpopulations have significant differences at the transcriptional level, and each expresses specific

marker genes. For example, the satellite cell population highly expresses stem cell markers such as PAX7, the myoblast population highly expresses myogenic regulatory factors such as MYOD1 and MYOG, and the FAPs population is enriched in stromal cell markers such as PDGFRA. Single-cell analysis can also reveal some rare or previously under-characterized cell types. For example, a small number of cells with adipocyte characteristics were identified in scRNA-seq of skeletal muscle of mice and pigs, suggesting that FAPs in muscle may differentiate into adipocytes under certain conditions. Similarly, there may be low-abundance but functionally important cell populations in goat muscle tissue that can only be identified by single-cell sequencing.

3.2 Gene expression patterns in muscle lineages

Single-cell transcriptomes can not only identify cell types, but also reveal the gene expression dynamics and regulatory networks of muscle lineage cells during differentiation. During muscle development, cells at different stages activate specific gene expression programs. By performing pseudotime analysis on single-cell data, the trajectory of cells from progenitor cells to differentiated cells can be reconstructed to observe when key genes are turned on or off. For example, in the trajectory of differentiation from myogenic precursors to myoblasts, cells in the early stage highly express PAX3/PAX7 and MYF5, representing the state of stem cells and primary progenitor cells; in the middle stage, factors that promote differentiation, such as MYOD1 and MEF2C, begin to be upregulated; and in the terminally differentiated myoblasts, muscle structural protein genes such as myosin heavy chain are significantly expressed (Zhou et al., 2022). Similar patterns are expected to be seen in single-cell data from goats. For example, assuming that along the trajectory from satellite cell activation to myoblast fusion, PAX7 will be highly expressed in the initial stage and decrease in the late stage, whereas MyoD/MyoG will be upregulated in the middle and late stages. This expression change has been confirmed in single-cell analyses of other species. At the same time, single-cell transcriptomes can also discover new genes or non-coding regulatory factors related to muscle development. Ye et al. (2023) found through transcriptome analysis that during the development of goat fetal muscle, the expression of structural-related genes such as integrin ITGA4/ITGA11 and troponin TNNT1/TNNT3 changed significantly, and participated in the regulation of myofilaments and adhesion plaques. The expression patterns of these genes revealed that they may play an important role in muscle formation. Although this study used bulk RNA-seq data, if single-cell sequencing is applied in the future, the expression of these genes can be further localized to specific cell subpopulations, thereby clarifying their role stages in the muscle lineage.

In addition to coding genes, single-cell analysis also revealed differences in the expression of non-coding RNAs in different muscle cells. For example, Han et al. (2022) used second-generation sequencing to compare the expression of lncRNAs in skeletal muscle at different developmental stages of goats, identified a series of lncRNAs related to muscle growth, and predicted through co-expression analysis that they may regulate muscle structure or metabolic genes. At the single-cell level, if certain lncRNAs are highly expressed in satellite cells and lowly expressed in differentiated muscle fibers, it indicates that they may play a role in the maintenance or activation of muscle stem cells. Similarly, Shen et al. (2022) sequenced small RNAs in skeletal muscle of goats of different breeds and found that multiple miRNAs were closely related to muscle growth and intramuscular fat deposition. For example, miR-133, miR-378, etc. were upregulated during the period of vigorous muscle growth, and the regulatory targets of these miRNAs involved myocyte proliferation and adipose differentiation. Combining these findings with single-cell transcriptome data can verify the distribution of these miRNAs in specific cell types and further clarify their cell background of action. Recently, a study on Wu'an goats reported that miR-665 was highly expressed in the muscles of 1-month-old young goats, and could target and inhibit the apoptosis-related gene *BCL2L11*, thereby promoting myoblast proliferation (Feng et al., 2025). This result provides an example of miRNA regulating muscle cell fate and reflects the impact of miRNA expression changes in developmental periods (juvenile vs. young) on muscle growth.

3.3 Key findings from transcriptome data

Using single-cell and other transcriptome data, researchers have made some important discoveries in skeletal muscle development in goats and related species. First, the regulatory mechanism of the balance between muscle and fat development has gradually become clear. Qiu et al. (2020) combined single-cell transcriptomics with

proteomics to reveal the important role of calcium ion signaling pathways in determining skeletal muscle growth potential. They found that there were significant differences in the expression of genes related to intracellular calcium homeostasis regulation in lean and obese pig muscle cells, suggesting that Ca2+ signals may be the key determinant of muscle tissue's choice of myogenesis or adipogenesis fate. This finding is also instructive for species such as goats, because the balance between intramuscular fat deposition and muscle fiber growth directly affects meat quality and meat production. Single-cell data helps us determine which signaling molecules mediate the interaction between muscle cells and fat precursors.

Secondly, the identification of key transcription factors is one of the important results of single-cell transcriptomics. Through comprehensive single-cell gene regulatory network analysis and functional verification, Zhu et al. (2024) identified the transcription factor *TCF7L2* as a central regulator in early adipogenesis in goat skeletal muscle. They not only inferred from the gene regulatory network of single-cell data that *TCF7L2* may control the differentiation of fat precursors, but also confirmed through in vitro intervention experiments that inhibiting *TCF7L2* would affect the differentiation process of adipocytes. This is a typical case of single-cell analysis guiding functional experiments, which shows that we can lock potential key factors from massive single-cell transcription information and then verify them at the cellular or animal level. In addition, there are similar experiences in bovine muscle research: for example, Zhang et al. (2020) found that a new lncRNA affects myogenesis by mediating the KRAS/Myf6 pathway. This discovery was also based on the analysis of transcriptome data and subsequent functional experiments. For goat muscle, due to the late start of genomic and transcriptional regulation research, the discovery of such key factors is particularly valuable, which provides specific targets for further genetic improvement.

Third, the combination of single-cell and bulk transcriptomes also revealed new regulatory molecules related to muscle development. In addition to the transcription factors such as *TCF7L2* mentioned above, the functions of many non-coding RNAs are also gradually being clarified. For example, Han et al. (2022) identified goat muscle lncRNAs that were differentially expressed at multiple developmental stages, some of which affected the proliferation and differentiation of muscle cells by competing with mRNA for the "sponge" effect of miRNA. Another study on goat fetal skeletal muscle found the involvement of natural antisense long-chain RNA (nat lncRNA) through transcriptome analysis. These natural antisense RNAs were negatively correlated with the expression of corresponding positive chain genes (such as muscle structural genes), and may regulate muscle development through chromatin modification or transcriptional interference mechanisms. In addition, in terms of small RNAs, Shen et al. (2022) compared skeletal muscle miRNAs of different goat breeds and found that some miRNAs (such as miR-432 and miR-133 families) were highly expressed in high intramuscular fat breeds, and it was speculated that they promoted adipogenesis and inhibited muscle fiber proliferation, thereby leading to higher intramuscular fat deposition.

4 Epigenetic Mechanisms of Muscle Development

4.1 Chromatin accessibility and histone modification

Epigenetic regulation plays a key role in muscle development by affecting gene expression without changing the DNA sequence. One important aspect is chromatin accessibility. The degree of chromatin openness determines whether transcription factors and transcription machinery can bind to DNA and initiate gene transcription. During the transition of muscle progenitor cells to differentiated muscle cells, the chromatin accessibility of related muscle gene promoters and enhancers will change significantly. For example, in a study of Hu sheep (Hu sheep), ATAC-seq (analysis of chromatin open regions) was compared on skeletal muscles of different ages, and 3742 differentially accessible regions were found to change during development (Cao et al., 2023). These open regions are significantly associated with genes related to muscle development pathways. For example, the open regulatory elements that appear at 3-6 months of age are enriched with transcription factor binding sites related to muscle hypertrophy and myofiber conversion, including ARID5B, MYOG, etc., which promote myofiber growth; while other open regions are enriched with binding sites of factors such as NR1D1 and ZFP36L2, which are related to myofiber type conversion. It can be seen that the dynamic changes in chromatin accessibility directly affect the fate determination of muscle cells. Through technologies such as single-cell ATAC-seq or 10x Multiome, the

transcriptome and chromatin openness can be measured simultaneously at the single-cell level, and then a certain open regulatory element can be linked to the gene expression of the cell in which it is located. This enables us to construct cell type-specific regulatory networks. For example, if the upstream enhancer of the MyoD promoter is specifically opened in satellite cells and closed in myoblasts, this suggests that the enhancer is essential for satellite cells to activate MyoD expression.

Another important level is histone covalent modification, such as acetylation and methylation, which are involved in determining the active state of chromatin. Histone acetylation is generally associated with transcriptional activation because acetylation neutralizes the positive charge of histones, weakens DNA-histone interactions, and makes chromatin loose and easy to transcribe. The metabolism and differentiation of skeletal muscle cells are regulated by the level of histone acetylation: Studies have shown that in muscle cells, the dynamic balance of histone acetyltransferases (HATs) and deacetylases (HDACs) precisely regulates the expression of related genes. For example, HDAC inhibitors can promote myoblast differentiation because they increase histone acetylation, thereby activating the transcription of muscle differentiation genes (Xu et al., 2023). Specific to certain landmark modifications, such as histone H3 lysine 9 acetylation (H3K9ac) and H3 lysine 27 acetylation (H3K27ac), they are often enriched in active gene promoters and enhancer regions, and can be used as a marker to identify active enhancers in muscle cells. In the mouse C2C12 myoblast induced differentiation model, H3K27ac increased significantly near the promoters of key genes such as Myogenin, corresponding to the rapid upregulation of genes. In contrast, histone deacetylation and specific histone methylation (such as H3K27 trimethylation) are high when muscle stem cells are maintained in an undifferentiated state, thereby silencing the expression of differentiation genes. This has been observed in satellite cells: H3K27me3 mediated by the polycomb complex (PRC2) is enriched at the muscle differentiation loci of quiescent satellite cells to inhibit premature differentiation; when satellite cells are activated, this modification decreases and the transcription of related genes is unrepressed.

A large amount of evidence shows that changes in histone modifications during muscle development are closely related to gene expression. For example, a review summarizes the effects of histone acetylation/deacetylation on skeletal muscle metabolism and phenotype, including regulation of myocyte cycle, fiber type conversion, muscle atrophy, and insulin sensitivity. For livestock and poultry such as goats, although there are not many studies on specific histone modification maps, similar mechanisms can be expected to exist. In addition, the role of histone methylation such as H3K4me3 (active mark) and H3K27me3 (repressive mark) in muscle development is also worthy of attention. For example, EZH2, as a methyltransferase of H3K27me3, plays a role in satellite cell self-renewal. A study detected high expression of EZH2 in some cells in single-cell sequencing of sheep spermatogonia (Xiong et al., 2022). It is speculated that EZH2 may also be involved in maintaining the stem cell state in muscle satellite cells. When EZH2 function is impaired, satellite cells may differentiate prematurely, deplete the stem cell pool, and lead to decreased muscle regeneration ability. On the contrary, if factors that regulate histone acetylation levels, such as HATs such as PCAF, are upregulated, they may promote the formation of muscle fibers.

4.2 DNA methylation patterns in muscle cells

DNA methylation is another important epigenetic mechanism, that is, adding methyl groups to cytosine in DNA (mainly occurring at CpG sites), which is usually associated with transcriptional repression. In skeletal muscle development, DNA methylation has a regulatory effect on cell fate determination and gene temporal expression. In general, high methylation in gene promoter/enhancer regions often leads to gene silencing, while demethylation is associated with gene activation. When muscle stem cells transform into differentiated cells, the methylation status of many muscle-specific gene promoters will change. For example, low methylation in the promoter regions of key myogenic factors such as MYOD1 and MYF5 is usually a prerequisite for their high expression, and literature has reported that the methylation level of these sites is negatively correlated with myogenic differentiation ability. A study on maternal malnutrition in goats directly demonstrated the effect of DNA methylation on muscle gene expression: when ewes were malnourished in mid-pregnancy, the methylation level of specific sites on the MYF5 promoter in the skeletal muscle of their fetuses increased significantly, resulting in decreased MYF5 expression; correspondingly, some sites of the MYOD promoter in the muscle of newborn lambs

also showed abnormal methylation (Zhou et al., 2022). These changes are associated with impaired muscle fiber development, indicating that external factors such as nutrition can affect goat muscle development through epigenetic pathways. The authors further pointed out that methylation in the promoter regions of genes such as MYF5 and MYOD is closely related to muscle growth and development, and its changes may mediate the developmental programming effects of nutritional stress on muscle.

The overall dynamics of DNA methylation are also important during normal development. High-throughput methylation sequencing (WGBS) has been used to analyze methylome changes in livestock skeletal muscle development. Ren et al. (2023) performed WGBS comparisons on the skeletal muscles of Hainan black goats and their hybrid offspring and found that the methylation distribution of the two strains was generally consistent across the genome, but there was differential methylation in specific gene regions, and these differences were associated with muscle growth performance. They identified thousands of differentially methylated regions (DMRs) and the genes involved, and combined RNA-seq to find 189 differentially expressed genes (DEGs), and finally locked in 11 hub genes that were both differentially methylated and differentially expressed. Among them, 9 genes were significantly associated with muscle growth traits (eye muscle area, muscle height and weight, etc.). In particular, the methylation and expression of the *PRKG1* gene were negatively correlated with muscle growth traits, that is, muscle growth slowed down when the methylation level increased and the expression decreased. In contrast, some FOX family transcription factors such as FOXO3 and FOXO6 have lower promoter methylation and higher expression in high growth performance groups, which help protect muscle fibers from stress and promote myotube differentiation. The study concluded that diverse DNA methylation modifications jointly maintain glycogen storage around muscle fibers by affecting key genes, thereby supporting the growth of muscle fibers. It can be seen that in livestock such as goats, differences in the methylation profile of the skeletal muscle genome under different strains or different developmental conditions will lead to differences in muscle growth phenotypes. This provides us with possible epigenetic markers for evaluating and improving the production performance of meat goats.

In addition to gene promoters, the pattern of DNA methylation in enhancers and other regulatory elements is also worthy of attention. The methylation of enhancers is usually negatively correlated with their activity. For example, in the study of sheep, some scholars found that some enhancer regions in muscle tissue are in a low methylation state, which is conducive to improving the expression of nearby genes and tissue function. If methylation data is combined with chromatin accessibility and gene expression through multi-omics integration, it is possible to locate those regions where changes in methylation status have the greatest impact on gene regulation. In the study of skeletal muscle development in Hu sheep, researchers found that those genomic regions that are both highly open and low in methylation are often combined with important transcription factors for muscle development, suggesting that the synergistic effect of DNA demethylation and chromatin opening promotes the activation of specific genes (such as MyoG, etc.). DNA methylation does not occur in isolation, but together with other epigenetic marks, it shapes the transcriptional program.

4.3 Epigenetic regulation of key muscle genes

By linking transcription and epigenetics, we can see specifically how several key muscle genes are regulated by epigenetic mechanisms. First, take the myogenic regulatory factor MyoD as an example: MyoD is one of the master switch genes that initiate myogenic differentiation, and the methylation state and histone modification of its promoter region directly affect its expression. In undifferentiated myogenic precursor cells, the MyoD promoter may be in a partially methylated state, accompanied by inhibitory histone marks, thus maintaining a silent state. Once the cells receive differentiation signals (such as reduced FGF signals), the activity of DNA methyltransferases decreases or TET protein-mediated demethylation increases, causing the MyoD promoter CpG site to be demethylated; at the same time, inhibitory modifications such as H3K27me3 decrease, and activating modifications such as H3K27ac increase, and the *MyoD* gene begins to transcribe, driving the cells into the differentiation program (Zhou et al., 2023). This epigenetic switch ensures that MyoD is expressed at the appropriate time and in the appropriate cell type.

Epigenetics also indirectly regulates muscle development by affecting key genes in signaling pathways. For example, the PI3K/Akt pathway is one of the main signaling pathways that promote muscle synthesis. A joint transcriptome and methylome analysis found that multiple components of the pathway showed a trend of low methylation and high expression in high-growth performance goats, such as AKT2 and ADCY5 genes, which had decreased methylation and increased expression in growth-advantaged individuals (Ren et al., 2023). The products of these genes enhance the promoting effect of insulin/IGF signals on muscle growth. On the contrary, some negative regulatory factors such as PTEN and PRKG1 have increased promoter methylation in high-growth individuals, resulting in reduced expression and relieving their inhibition of the PI3K/Akt pathway. It can be seen that the epigenetic modification state of key signaling genes is closely related to the rate of muscle growth. By artificially intervening in epigenetic states (such as using DNA methylation inhibitors or HDAC inhibitors), these effects may be simulated to a certain extent, thereby adjusting the balance between muscle generation and degradation. However, before being applied to production practice, its safety and long-term effects need to be carefully evaluated.

In addition to DNA and histone modifications, RNA epigenetics (i.e., epitranscriptome) has become a hot topic in recent years. Among them, N6-methyladenine (m6A) modification is one of the most common mRNA modifications, which also affects muscle development. Research by Wang Feng's team at Nanjing Agricultural University revealed that the m6A demethylase FTO can promote goat myoblast differentiation by reducing the m6A level of GADD45B mRNA: when FTO demethylates the GADD45B transcript, the *GADD45B* gene is efficiently expressed, thereby activating the p38 MAPK signaling pathway and promoting myogenic differentiation. Knocking down FTO blocks this process and inhibits myoblast differentiation (Su et al., 2025). This finding belongs to epigenetic regulation at the RNA level, indicating that mRNA modification is also involved in the fine regulation of muscle development. For livestock such as goats, the regulatory role of m6A modification may be quite extensive, because many development-related mRNAs have m6A marks that regulate their splicing, stability, and translation efficiency.

Finally, the plasticity of epigenetic regulation should be pointed out. Unlike gene sequences, epigenetic marks can be changed by environmental and physiological conditions. For example, maternal malnutrition, as mentioned above, affects fetal muscle gene methylation. Exercise, stress, and disease may also change the epigenetic state of muscle cells through hormones and inflammatory mediators. This plasticity means that through nutrition, exercise, and other means, it may be possible to optimize the epigenetic state of livestock muscle development to a certain extent. For example, studies have found that moderate exercise stimulation can change the histone acetylation of some metabolic gene promoters in skeletal muscle, increase their expression, and enhance the metabolic capacity of muscle (Zheng et al, 2025).

5 Application of Multi-Omics Integration in the Discovery of Regulatory Mechanisms

5.1 Integration of transcriptome and epigenomic data

Data from a single omics can often only reflect one aspect of a biological process, while multi-omics integration can link information at different levels to build a more complete regulatory picture. In the study of skeletal muscle development, it is often necessary to combine transcriptome data with epigenetic data to determine which epigenetic changes lead to changes in gene expression. A typical example is the aforementioned study of Hainan black goats: by integrating WGBS methylation data with RNA-seq expression data, researchers screened out 11 key genes from thousands of differentially expressed genes. These genes showed both differential methylation and differential expression, and were therefore identified as important regulators affecting muscle growth. This association analysis greatly narrowed the candidate range and improved the efficiency of discovering key factors. Similarly, if we have multi-omics data at the single-cell level (such as ATAC-seq and RNA-seq of the same cell), we can more directly establish a one-to-one connection between genes and regulatory elements. For example, Ranzoni et al. (2021) integrated and analyzed single-cell RNA and ATAC data of human hematopoiesis and constructed a network map of genes and potential regulatory elements during development. In this network, they identified the correlation between the opening of binding sites of certain transcription factors and the expression of downstream genes, thereby discovering new regulatory axes. Similarly, for goat muscle, if ATAC and RNA

integration can be performed at the single-cell level, we can find relationships such as "enhancer X opened in satellite cells promotes the expression of myogenic gene *Y*". This information is crucial for proving causal relationships. At present, even in the absence of simultaneous sequencing data of single-cell multi-omics, we can also use the method of single-cell clustering + omics matching: first use single-cell transcriptomes to divide cell types, then perform ATAC-seq or ChIP-seq analysis on the corresponding cell groups, and finally match the two. The study of Hu sheep skeletal muscle development used a similar method to superimpose the ATAC accessible regions and low-methylation regions of muscles at a specific stage, and discovered a transcription factor network related to muscle development (Cao et al., 2023).

5.2 Computational tools for multi-omics analysis

The complexity of multi-omics data requires advanced computational tools and algorithms for processing and integration. Commonly used single-cell multi-omics analysis frameworks include Seurat, Signac, Scanpy, etc., which have supported joint dimensionality reduction and clustering analysis of single-cell transcriptome and ATAC-seq data. For example, Seurat can use the "anchoring" method to align the data of cells in different modalities, thereby identifying the corresponding cell types across omics. There are also some specialized tools for associating regulatory elements with genes, such as the Cicero algorithm, which can predict co-open chromatin regions based on single-cell ATAC data and infer possible enhancer-promoter connections. For the integration of DNA methylation and transcriptome, existing algorithms can perform correlation analysis on methylation levels and gene expression, and combine genomic annotations to determine whether methylation occurs on promoters, gene bodies, or distal regulatory elements. For example, Ren et al. (2023) used weighted gene co-expression network analysis (WGCNA) and other methods to screen hub genes when analyzing goat methylation and expression. Such network analysis integrates multi-omics data into the same association network model, and finds the intersection gene set by calculating the association between gene expression modules and epigenetic feature modules.

Another important aspect is visualization and database support. Today, there are some public databases, such as WashU Epigenome Browser and UCSC Genome Browser, which allow different types of data to be superimposed and plotted to intuitively display, for example, "ATAC signals, H3K27ac signals and DNA methylation levels of the promoter region of a gene under different conditions." This is very helpful for interpreting multi-omics results. In addition, machine learning technology has also begun to be applied to multi-omics integration. For example, deep learning models can combine DNA sequences, epigenetic modifications and gene expression for prediction and find new regulatory sequence patterns. It is worth noting that single-cell multi-omics particularly needs to solve the problems of data sparsity and noise. Because the molecules available to a single cell are very limited, such as only a small number of accessible regions are detected in each cell in scATAC, and many genes in scRNA have many zero values. This poses a challenge to integration. To this end, some tools use the "pseudocell" aggregation method to first merge similar cell data to improve the signal-to-noise ratio, and then perform omics association. In addition, there are statistical models such as Liger and MOFA that can perform multi-omics fusion in the presence of missing data. Liger extracts common and specific factors through non-negative matrix decomposition, and MOFA uses factor analysis models to find potential factors that drive changes in different omics. These methods are constantly being improved and applied to new biological scenarios.

5.3 Advantages and limitations of multi-omics in muscle research

Multi-omics integration has shown significant advantages in skeletal muscle development research. First, it provides a more comprehensive perspective, which can consider multiple levels of gene regulation at the same time, avoiding one-sided conclusions caused by looking at a certain level in isolation. For example, if we only look at the transcriptome, we may find that a gene is upregulated, but we don't know the reason; and combined with methylation data, we may find that it is activated by promoter demethylation, thereby elevating the discovery to a mechanistic level of understanding (Zhou et al., 2022). Second, multi-omics integration improves the credibility of the signal. Different omics data are mutually verified, which can reduce false positives. For example, if a gene is identified as differentially expressed and its upstream regulatory elements also have significant epigenetic changes, we are more confident that it does have biological significance. Thirdly, at the single-cell level,

integration can reveal the association of intracellular events. For example, a cell may change the expression of a series of downstream genes due to an epigenetic mutation (such as abnormal chromatin opening), which is easy to be diluted or misunderstood in bulk data, but can be accurately captured in single-cell multi-omics.

Of course, multi-omics methods also have limitations. The first is technical complexity and cost. The current single-cell platforms that can detect multiple omics simultaneously (for example, 10x Multiome can measure RNA and ATAC simultaneously) are expensive and have high sample requirements. For tissues of large animals (such as adult goats), it is challenging to prepare single cell nuclei freshly and keep different omics molecules intact. In addition, simultaneous multi-omics often results in a decrease in the data quality of each omics compared to single sequencing. For example, the number of genes and open regions that can be detected in the same cell are less than the level when they are sequenced separately. Secondly, data analysis is more difficult. It is necessary to deal with high-dimensional and high-sparse data, and design effective statistical tests to support the inference of causal associations between multiple omics. Some integrated conclusions may still be relevant and require additional experimental verification. Third, data standardization and control are a problem. Different omics have different dynamic ranges and noise characteristics, and it is difficult to interpret their changes at the same scale. Multi-omics research also requires interdisciplinary collaboration, and bioinformatics analysts and experimental biologists need to work closely together to interpret the data.

6 Case study: Application of Single-Cell Multi-Omics in Muscle Development Research

6.1 Representative studies in livestock muscle research

Single-cell multi-omics has many successful cases in model organisms such as mice and humans, and has also begun to emerge in livestock muscle research. In 2024, Zhu et al. (2024) reported the first single-nuclear transcriptome map of goat skeletal muscle development, focusing on the regulation of adipogenesis in muscle. They selected goat longissimus dorsi muscles at different developmental stages for single-nuclear RNA sequencing, and obtained transcriptional expression profiles of up to thousands of nuclei. Through cluster analysis, the study identified several major subpopulations in goat skeletal muscle, including muscle satellite cells, myoblasts, fibroblast/adipocyte progenitor cells (FAPs), and immune cells. Of particular concern is that FAPs are subdivided into several subpopulations, one of which is enriched in adipogenic marker genes (such as PPARγ), indicating that it is on the path of differentiation into adipocytes. By constructing a ligand-receptor interaction network, the authors found that there are significant signal connections between FAPs and muscle satellite cells, such as the BMP pathway and the IGF pathway, which means that FAPs may affect satellite cells and myoblasts by secreting factors such as BMP and IGF, thereby regulating the balance between muscle and adipocytes. More importantly, the authors used single-cell gene regulatory network analysis to predict and verify the key role of *TCF7L2* transcription factor in early intramuscular fat production. Single-cell data suggested that *TCF7L2* may regulate a network of multiple fat-related genes. They then knocked down *TCF7L2* in primary cells, and the result was that fat differentiation was inhibited, confirming this inference. This is a rare study in large animals that discovered and functionally verified transcription factors by single-cell means. This case fully reveals the relationship between myocytes and adipocyte precursors in goat muscle tissue, and finds out the key factors that affect meat quality (fat deposition), which has direct significance for animal husbandry. For example, *TCF7L2* can be regarded as a large candidate gene that affects goat meat quality, and future breeding can focus on whether its allele variation is associated with meat quality traits.

6.2 Insights from epigenetic and transcriptional regulation

Muscle-fat fate is determined by a complex signaling network, and single-cell technology has helped to dismantle this network. The traditional view is that muscle and fat originate from different cell lineages and do not interfere with each other. However, recent single-cell studies have broken this dualism and revealed that there is a close interaction and balance between myogenic cells and adipocyte precursors within muscle tissue. For example, studies in pigs and goats have pointed out that when signals that promote myogenesis are dominant (such as active pathways such as IGF and Ca^{2+}), muscle growth is dominant; conversely, when factors that promote adipogenesis (such as PPAR signals) are enhanced, intramuscular fat deposition increases and muscle fiber growth is restricted (Qiu et al., 2020; Zhu et al., 2024). This balance is influenced by both genetics and the environment. At the

molecular level, the role of the transcription factor *TCF7L2* is a brilliant example. As a downstream factor of the Wnt signaling pathway, it was found to be a switch that favors adipogenic differentiation. When *TCF7L2* activity is high, FAPs are more likely to turn to adipocytes, thereby increasing intramuscular fat; when its activity is inhibited, adipose differentiation is weakened, and more cells may retain myogenic potential.

Epigenetic reprogramming accompanies muscle development and responds to external conditions plastically. Muscle cells of different types or stages have significantly different transcriptional states, and there must be epigenetic changes behind these state changes. For example, in lean pig muscle cells, the chromatin of myogenesis-related genes may be more open, while in obese pigs, fat-related genes are more open (Qiu et al., 2020). If further ATAC or ChIP analysis is performed, this is expected to be verified. Similarly, when goat satellite cells are activated into myoblasts, some key gene promoters are demethylated and H3K27ac increases, which are expected epigenetic changes. Therefore, these transcriptional changes essentially reflect epigenetic reprogramming. And importantly, this reprogramming is plastic.

Interactions across cell types are an important level of muscle development regulation. Traditionally, muscle biology has focused on the differentiation regulation of muscle cells themselves (myoblasts, myofibers). However, the case emphasizes the impact of fibroblast/adipocyte progenitor cells (FAPs) on the muscle environment (Zhu et al., 2024). FAPs have long been regarded as the culprits of scar formation and fat infiltration in regenerative medicine, but single-cell studies have found that they also provide necessary matrix support in normal development. Muscle tissue is not an isolated island of muscle cells. It forms a unified niche together with surrounding connective tissue, nerves, and immune cells. Single-cell multi-omics allows us to observe these different cells at the same time, so as to understand, for example, how cytokines secreted by immune cells affect satellite cells and how ECM production by fibroblasts regulates the mechanical environment of muscle fibers. This is indistinguishable in traditional bulk sequencing. Taking goat muscle as an example, if we also introduce spatial transcriptomics or multi-omics in the future, we can directly see the relationship between the location of immune cell aggregation and muscle fiber growth on tissue sections, thereby taking cell interaction research a step further.

6.3 Implications for future research and animal husbandry improvement

On the one hand, in scientific research, the case demonstrates the power and necessity of single-cell multi-omics. In the past, many genetic studies on muscle growth were unable to clarify cellular heterogeneity and multi-layer regulation due to the lack of single-cell resolution, so that some conclusions remained at the correlation level. Now that we have these technologies, we should vigorously apply them to livestock research. For example, we can conduct systematic single-cell transcriptome and epigenomics analysis on goat skeletal muscle of different breeds, different growth stages, and different nutritional conditions to establish a multi-omics reference map of goat skeletal muscle development. On this basis, further functional experiments can be more targeted around key genes and pathways, thereby accelerating the output of results. On the other hand, for animal husbandry practice, these studies provide new ideas for genetic improvement and feeding management (Zhou et al., 2024). In terms of genetic improvement, the core regulatory factors and pathways found by single-cell multi-omics can be converted into molecular markers for breeding. For example, traditional breeding indicators (weight gain, backfat thickness, intramuscular fat content, etc.) can be combined with molecular markers to improve selection accuracy. The rise of gene editing technology also gives us the opportunity to directly modify these factors. If the role of a certain factor is critical and has no obvious adverse effects (such as knocking out the inhibitory factor in muscle), in theory, gene editing can be used to create a new strain with more ideal muscle growth. Of course, the application of gene editing in production still faces ethical and regulatory challenges, but it is technically feasible, such as the creation of sheep without muscle growth inhibitor MSTN has been reported (Figure 2) (Wang et al., 2017). Single-cell multi-omics will tell us that a more delicate regulatory network than MSTN allows us to combine multiple means to achieve breeding goals.

In terms of feeding management, after understanding the regulatory mechanism of muscle development, we can design refined nutritional strategies or environmental interventions to optimize the phenotype. For example, if it is

found that moderate exercise can increase the diameter and oxidative metabolic capacity of muscle fibers through epigenetic pathways, then the farm can consider providing meat goats with a certain amount of activity space or free-range feeding to improve meat quality. Nutrition such as specific vitamins and trace elements can affect the activity of DNA/histone modifying enzymes and can also be used as an adjustment strategy. For example, the levels of methyl donors such as choline and folic acid affect DNA methylation; ketoacids and niacin affect histone acetylation; these can all be taken into account in feed formulation in order to optimize the epigenetic regulatory environment for muscle growth. Of course, this requires a lot of experiments to verify the effect.

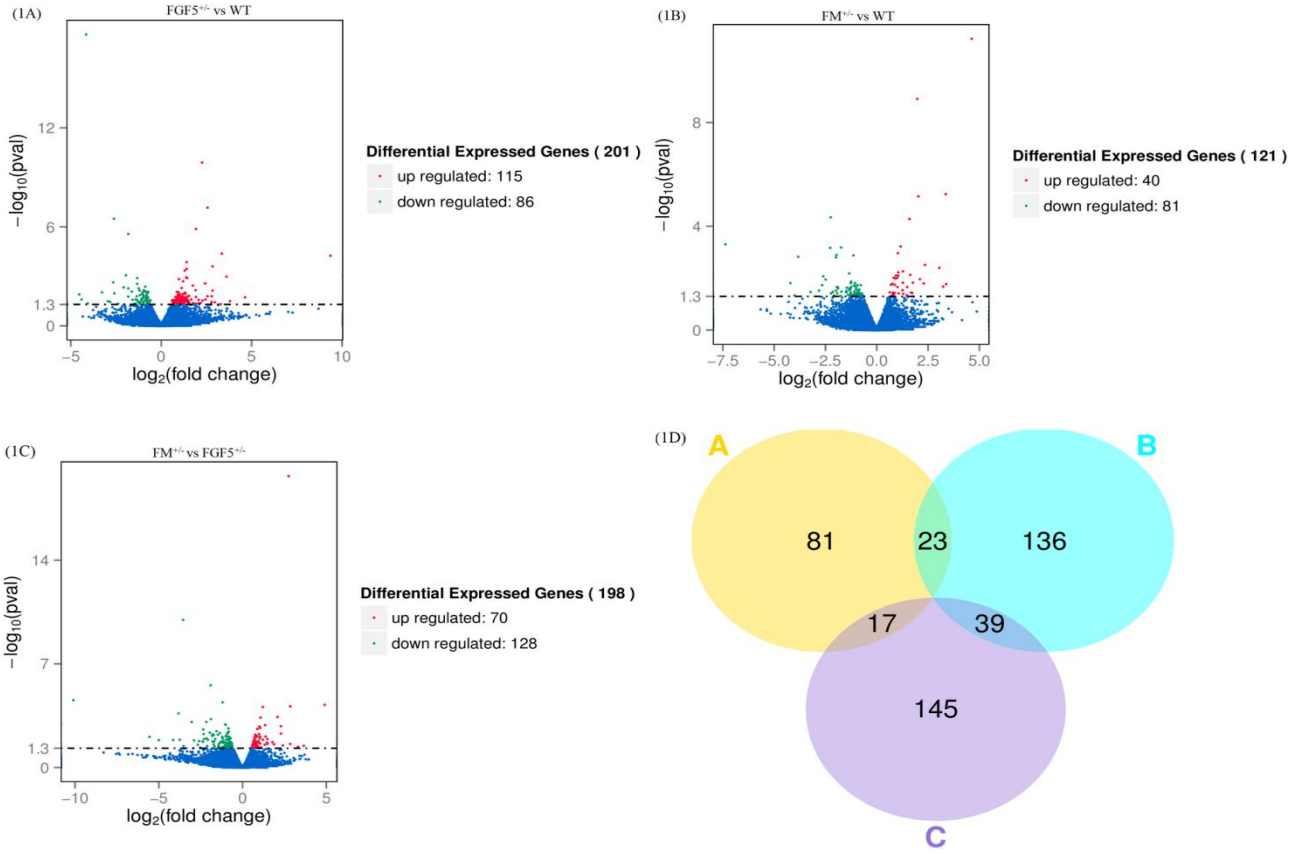

Figure 2 Differentially expressed genes in the RNA-Seq data (Adopted from Wang et al., 2017)

Image caption: Volcano plot of statistically significant differentially expressed genes at $P \leq 0.05$ identified from the RNA-Seq libraries of normal and transgenic goat muscle (1A, 1B, 1C). A Venn diagram showing the DEGs identified from comparisons of FGF5 vs WT goats, FM vs WT goats and FM vs FGF5 goats (1D) $^{+/-+/-+/-}$ (Adopted from Wang et al., 2017)

7 Future Prospects and Challenges

7.1 Expanding the application of multi-omics in livestock

Single-cell multi-omics has shown great potential in livestock muscle research, and there are several major directions for expansion in the future. The first is the expansion of the scope of application: in addition to skeletal muscle development, this technology can also be applied to the study of other important tissues and traits of livestock, such as metabolic regulation of adipose tissue, mammary gland development and lactation, and germ cell development. In fact, Chinese scholars have used single-cell transcriptomes for research on goat testicular development and sheep wool follicle development, and have achieved breakthrough insights (Xiong et al., 2022). These successful experiences show that it is feasible and valuable to conduct single-cell omics research on livestock species. In terms of skeletal muscle, research topics can be further refined in the future, such as: single-cell omics changes during muscle injury repair (to explore ways to improve muscle regeneration ability), single-cell maps of developmental differences in different muscle parts (leg muscles vs. longissimus dorsi), and sex differences in muscle development (male and female livestock). These will provide more specific guidance for breeding. For example, if it is found that the slow growth of leg muscles in a certain breed is due to specific signals that limit the activity of satellite cells, targeted improvements can be made during breeding.

The second is the expansion of multimodal omics. Current research focuses on transcriptomes, DNA methylation, chromatin accessibility, etc. In the future, single-cell data such as proteomes, epitranscriptomes (such as m6A maps), and metabolomes can be further introduced. In particular, single-cell proteomics and spatial omics are two cutting-edge directions. Single-cell proteomics technology is developing. Once mature, we can directly measure the protein expression profile of a single muscle cell, which is critical for verifying the results of transcriptional regulation and revealing post-translational regulation (Qiu et al., 2018). Spatial omics can obtain molecular information at different locations while maintaining tissue structure, which is extremely useful for organically connected tissues such as muscles. For example, spatial transcriptomes can tell us whether there are different expression programs in cells in the center and edges of muscle bundles, and whether there are metabolic differences between muscle fibers around capillaries and those in the middle. This information could only be inferred through histology in the past, but now it is expected to be quantitatively obtained through omics methods.

Cross-species comparison is also a direction for expansion. Single-cell omics data of livestock can not only be compared with model animals to find commonalities and specificities; different livestock species can also be compared with each other to learn from each other. For example, goats and sheep are evolutionarily close and have similar production uses. If the differences in their muscle development omics characteristics can be compared, it may explain why the meat quality and fat deposition patterns of sheep are different from those of goats. This comparative study can also help transfer knowledge. For example, a key lncRNA regulatory mechanism found in sheep may also exist in goats, but the gene sequence is slightly different, and the corresponding sequence function needs to be located.

7.2 Converting research results into genetic improvement

How to truly apply cutting-edge research results to genetic improvement is an important issue facing scientists and breeding experts. There is a conversion path from single-cell multi-omics to breeding practice, which requires overcoming some obstacles and adopting innovative strategies. First, it is necessary to screen key markers for stable inheritance. Many regulatory mechanisms discovered by single-cell omics are epigenetic or environmental related, and not all are directly determined by DNA sequences. Therefore, to be used in breeding, DNA markers (SNPs, InDels, CNVs, etc.) associated with these mechanisms must be found. For example, as mentioned above, the regulatory effect of *TCF7L2* on intramuscular fat is clear, so the next step is to study the allelic variation of the *TCF7L2* gene in the goat population to see if there are any mutations that affect its function or expression. If so, use it as a selection marker; if there is no significant variation, the regulation of *TCF7L2* may be more at the level of regulatory regions (such as enhancers), and then genetic variations in upstream regulatory genes of *TCF7L2* (such as other components of the Wnt pathway) can be sought. For example, miR-665 promotes myogenesis. We can check the sequence differences of the *miR-665* gene or its target site *BCL2L11* gene in different varieties to see if there are any mutations associated with muscle mass traits (Feng et al., 2025). This conversion from mechanism to marker is very labor-intensive, but it is a necessary step. Fortunately, the mechanistic knowledge provided by single-cell omics can significantly narrow the range of genes we focus on, making association analysis more targeted.

Secondly, we can consider the application of new technologies such as gene editing in breeding. Traditional breeding accumulates favorable alleles through multiple generations of selection, while gene editing can introduce target mutations in one step. For example, if a DNA methylation hotspot region (an enhancer that regulates a certain inhibitory factor) is confirmed to have a negative impact on muscle growth, we can try to edit the region through CRISPR/Cas9 to weaken its activity and achieve a similar effect to transgenics, but in fact, it is only editing the regulatory elements rather than adding exogenous genes. This type of operation may be easier than introducing exogenous genes in terms of legal and social acceptance. Chinese scientists have bred sheep with a knockout of the myogenin gene, which significantly increased skeletal muscle mass (Wang et al., 2017).

Third, computer breeding and simulation will become more important. As more and more data is obtained, computational models can be developed to predict how muscle development will change if certain gene expressions are changed. Such models can be trained with single-cell multi-omics data. Once the model is reliable,

we can test various hypotheses on the computer, such as "what happens if the activity of gene *X* is increased by 10%" and "what happens if the function of gene *Y* is deleted", and select the best improvement plan from them, and then verify it in real animals. This can save a lot of time and cost, and avoid unnecessary use of animals caused by blind trial and error.

7.3 Ethical, technical and practical considerations

On the road to improving livestock using single-cell multi-omics and genetic engineering, we must also face some ethical, technical and practical challenges. Gene editing of animals and in-depth analysis of animal embryonic development may cause public concerns about animal welfare and biosafety. The general public may question whether such in-depth cell manipulation of animals will cause pain or unnatural changes to animals. Therefore, when promoting these studies and applications, we need to be transparent and open, actively carry out popular science communication, clarify the purpose and safety of the technology, and let the public understand that improving animal production performance does not mean "abuse" or "monsterization" of animals. The animals themselves will not suffer as a result, but may be healthier (such as reducing diseases or growth stagnation). At the same time, we must respect certain ethical bottom lines, such as not blindly breeding animals with high artificial intervention in large quantities in the natural environment to avoid impacting the ecology.

Single-cell multi-omics still has room for improvement. As mentioned above, there are problems such as cost and high data complexity. This requires us to establish a cross-disciplinary cooperation team to introduce the latest sequencing technology and analysis algorithms into animal husbandry research. Interdisciplinary training is also necessary, so that traditional breeding experts can learn computational and molecular tools, and data scientists can understand biological problems. Technical details such as how to separate high-quality nuclei from meat livestock muscles with high fibroblast content, how to ensure the activity of samples transported over long distances, and how to deal with sample heterogeneity (such as batch effects of different individuals) need to be explored and solved. Perhaps several regional single-cell sequencing centers can be established to serve surrounding agricultural research units, focus on solving technical problems, and provide standardized services.

In addition to the technology itself, the acceptance of farmers, economic benefits, and regulatory policies should also be considered when applying scientific research results to the breeding industry. New molecular breeding technologies may require certain investments, training, and strategic planning. For example, single-cell multi-omics-assisted breeding can be piloted in national core breeding farms first, and then gradually promoted after significant results are achieved. At the same time, corresponding industry standards and regulatory frameworks should be formulated, such as the identification, detection methods, and risk assessment systems of gene-edited animals. At present, the international regulation of gene editing of livestock and poultry is different. The United States has conditionally released related products from Japan and China, and they are also under discussion. We need to improve policies based on scientific evidence to encourage innovation and ensure safety.

There are also issues of data ownership and privacy. Although animals do not have the concept of personal privacy like humans, breed resources belong to national or corporate assets. How to share and protect large-scale animal genetic and epigenetic data requires policy guidance. Perhaps a national livestock and poultry single-cell genomics database can be established, with data uploaded by various scientific research units, managed in a unified manner, and open for use in a graded manner. This can not only accelerate research progress, but also avoid data fragmentation and duplication of work. Finally, animal welfare must be mentioned. Any biotechnology method should be implemented without reducing animal welfare, and even with the goal of improving animal health and well-being. For example, if genetic improvement can make animals more resistant to disease and less prone to illness, this is a good thing for the animals themselves and should be strongly promoted. On the contrary, if a certain improvement leads to an increase in the physiological burden of the animal (such as extreme muscle development leading to skeletal and cardiac load), it needs to be treated with caution and production should not be sacrificed at the expense of animal health.

Acknowledgments

The authors thank the two anonymous peer reviewers for their thorough review of this study and for their valuable suggestions for improvement.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

An Q., Wang X., Song X., Meng J., Zhao Y., and Wu Z., 2022, Identification of up-regulated genes related to muscle growth and development in Guizhou white goats based on transcriptome analysis, Chinese Journal of Animal Science, 11: 157-163.

Cao Y., Ai Y., Zhang X., Zhang J., Long X., Zhu Y., Wang L., Gu Q., and Han H., 2023, Genome-wide epigenetic dynamics during postnatal skeletal muscle growth in Hu sheep, Communications Biology, 6(1): 1077.
https://doi.org/10.1038/s42003-023-05439-0

Deng K., Fan Y., Liang Y., Cai Y., Zhang G., Deng M., Wang Z., Lu J., Shi J., Wang F., and Zhang Y., 2021, FTO-mediated demethylation of GADD45B promotes myogenesis through the activation of p38 MAPK pathway, Molecular Therapy-Nucleic Acids, 26: 34-48.
https://doi.org/10.1016/j.omtn.2021.06.013

Feng J., Sheng H., Zhang X., Guo X., Yao D., Li Y., Chen L., and Zhang J., 2025, miR-665 targeting BCL2L11 regulates the myoblasts proliferation of Wu'an goat, Acta Veterinaria et Zootechnica Sinica, 56(2): 582-590.

Han H., Wang X., Li W., Liu J., Fan Y., Zhang H., Yang J., Gao Y., and Liu Y., 2022, Identification and characterization of lncRNAs expression profile related to goat skeletal muscle at different development stages, Animals, 12(19): 2683.
https://doi.org/10.3390/ani12192683

He J., and Li J., 2024, Observation analysis of embryonic development genes in high fertility goat breeds, Animal Molecular Breeding, 14(4): 271-279.
http://dx.doi.org/10.5376/amb.2024.14.0028

Qiu K., Xu D., Wang L., Zhang X., Jiao N., Gong L., and Yin J., 2020, Association analysis of single-cell RNA sequencing and proteomics reveals a vital role of Ca^{2+} signaling in the determination of skeletal muscle development potential, Cells, 9(4): 1045.
https://doi.org/10.3390/cells9041045

Qiu K., Zhang X., Wang L., Jiao N., Xu D., and Yin J., 2018, Protein expression landscape defines the differentiation potential specificity of adipogenic and myogenic precursors in the skeletal muscle, Journal of Proteome Research, 17(11): 3853-3865.
https://doi.org/10.1021/acs.jproteome.8b00530

Qiu X., Mao Q., Tang Y., Wang L., Chawla R., Pliner H.A., and Trapnell C., 2017, Reversed graph embedding resolves complex single-cell trajectories, Nature Methods, 14(10): 979-982.
https://doi.org/10.1038/nmeth.4402

Ranzoni A.M., Tangherloni A., Berest I., Riva S.G., Myers B., Strzelecka P.M., Xu J., Panada E., Mohorianu I., Zaugg J.B., and Cvejic A., 2021, Integrative single-cell RNA-seq and ATAC-seq analysis of human developmental hematopoiesis, Cell Stem Cell, 28(3): 472-487.
https://doi.org/10.1016/j.stem.2020.11.015

Ren Y., Chen X., Zheng X., Wang F., Sun R., Wei L., Zhang Y., Liu H., Lin Y., Hong L., Huang X., and Chao Z., 2023, Diverse WGBS profiles of longissimus dorsi muscle in Hainan black goats and hybrid goats, BMC Genomic Data, 24(1): 77.
https://doi.org/10.1186/s12863-023-01182-x

Shen J., Hao Z., Luo Y., Zhen H., Liu Y., Wang J., Hu J., Liu X., Li S., Zhao Z., Liu Y., Yang S., and Wang L., 2022, Deep small RNA sequencing reveals important miRNAs related to muscle development and intramuscular fat deposition in Longissimus dorsi muscle from different goat breeds, Frontiers in Veterinary Science, 9: 911166.
https://doi.org/10.3389/fvets.2022.911166

Su Y., Deng K., Liu Z., Zhang Z., Liu Z., Huang Z., Gao Y., Gao K., Fan Y., Zhang Y., and Wang F., 2025, m6A modified pre-miR-503-5p contributes to myogenic differentiation through the activation of mTOR pathway, International Journal of Biological Macromolecules, 294: 139517.
https://doi.org/10.1016/j.ijbiomac.2025.139517

Wang Q, Wang Z, Zhang Z, Li C., Zhang M., Ye Y., Wang S., and Jiang K., 2020, Overview of the technology of single cell sequencing, Chinese Journal of Medicinal Guide, 22(7): 433-439.

Wang L., Cai B., Zhou S., Zhu H., Qu L., Wang X., and Chen Y., 2017, RNA-seq reveals transcriptome changes in goats following myostatin gene knockout, PloS One, 12(12): e0187966.
https://doi.org/10.1371/journal.pone.0187966

Wu T., Wang S., Wang L., Zhang W., Chen W., Lv X., Li Y., Hussain Z., and Sun W., 2020, Long noncoding RNA (lncRNA) CTTN-IT1 elevates skeletal muscle satellite cell proliferation and differentiation by acting as ceRNA for *YAP1* through absorbing miR-29a in Hu sheep, Frontiers in Genetics, 11: 843.
http://dx.doi.org/10.3389/fgene.2020.00843

Xiong H., Sha Q., Liu S., Xiang D., Zhang B., and Zhao Z., 2022, Application of single-cell transcriptome sequencing in animals, Biotechnology Bulletin, 38(3): 226-233.

Xu J., Li C., and Kang X., 2023, The epigenetic regulatory effect of histone acetylation and deacetylation on skeletal muscle metabolism-a review, Frontiers in Physiology, 14: 1267456.
https://doi.org/10.3389/fphys.2023.1267456

Ye J., Deng M., Xue H., Liu G., Zou X., Sun B., Guo Y., Liu D., and Li Y., 2023, Identification and analysis of mRNA and lncRNA affecting goat fetal muscle development, Acta Veterinaria et Zootechnica Sinica, 54(3): 989-1002.
https://doi.org/10.11843/j.issn.0366-6964.2023.03.013

Zhang X., Chen M., Liu X., Zhang L., Ding X., Guo Y., Li X., and Guo H., 2020, A novel lncRNA, lnc403, involved in bovine skeletal muscle myogenesis by mediating KRAS/Myf6, Gene, 751: 144706.
https://doi.org/10.1016/j.gene.2020.144706

Zheng X., Liu X., Guo Y., Lv Y., Lin C., Wang D., Wang S., Liu Y., and Hu X., 2025, Physical exercise and epigenetic modifications in skeletal muscle, brain, and heart, Epigenetics & Chromatin, 18(1): 12.
https://doi.org/10.1186/s13072-025-00576-8

Zhou Q.Q., Lui H., and Huang S.Q., 2024, Advances in goat disease resistance through genetic selection, International Journal of Molecular Veterinary Research, 14(5): 211-219.
http://dx.doi.org/10.5376/ijmvr.2024.14.0024

Zhou X., Yan Q., Liu L., Chen G., Tang S., He Z., and Tan Z., 2022, Maternal undernutrition alters the skeletal muscle development and methylation of myogenic factors in goat offspring, Animal Bioscience, 35(6): 847.
https://doi.org/10.5713/ab.21.0285

Zhu J., Huang L., Zhang W., Li H., Yang Y., Lin Y., Zhang C., Du Z., Xiang H., and Wang Y., 2024, Single-nucleus transcriptional profiling reveals *TCF7L2* as a key regulator in adipogenesis in goat skeletal muscle development, International Journal of Biological Macromolecules, 281: 136326.
https://doi.org/10.1016/j.ijbiomac.2024.136326

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