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Integrative transcriptomic and metabolomic analyses reveal the role of melatonin in promoting secondary hair follicle development in cashmere goats

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Abstract

Background Melatonin improves the production performance of animal furs, particularly in promoting wool and cashmere growth. Although most studies of melatonin enhancing cashmere growth have focused primarily on gene and phenotype levels, its impact on metabolites has not received attention. To investigate the influence of melatonin on metabolites, genes, gene–metabolite interactions, and associated signaling pathways in secondary hair follicles (SHFs), we performed multiomics analyses of skin and blood samples collected 30 days after sustained melatonin release.

Results The results demonstrated that two melatonin interventions during SHF anagen in cashmere goats induce the early growth of SHFs, increase the active secondary follicle density (ASFD), and improve cashmere yield and quality. Transcriptomic analysis revealed 509 differentially expressed genes (DEGs), including key genes such as *KRT*s and *KRTAP***s**, and genes associated with the WNT signaling pathway (*LEF1*, *WNT3/4*, and *FZD3/5*), suggesting their critical roles in melatonin-mediated SHF development. Metabolomic analysis revealed 842 metabolites in the skin samples and 1,162 in the blood samples. Among these, 177 differentially regulated metabolites (DRMs) in the skin were significantly enriched in pathways such as alpha-linolenic acid metabolism, glyoxylate and dicarboxylate metabolism, the citrate cycle (TCA cycle), and several amino acid metabolic pathways. Similarly, 122 DRMs in the blood were enriched in pathways related to protein digestion and absorption, central carbon metabolism in cancer, and aminoacyl-tRNA biosynthesis. Finally, the integrative analysis revealed partially coenriched metabolic pathways and relationships between DEGs and DRMs.

Conclusions In summary, by integrating transcriptomics and metabolomics, this study provides novel insights into the role of melatonin in promoting SHF development. Furthermore, these findings establish a theoretical foundation for the broader application of melatonin-based technologies to promote cashmere growth.

Keywords Melatonin, Cashmere goat, Secondary hair follicle, Transcriptomics, Metabolomics

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Introduction

Cashmere, the most economically valuable product of cashmere goats, is a premium raw textile material that is often celebrated as "fiber gem" or "soft gold" [1]. Currently, China, Mongolia, Iran, and Afghanistan are the primary regions for cashmere production, with China and Mongolia collectively accounting for more than 90% of the global cashmere output [2]. However, the individual yield and total production of cashmere goats across various countries remain low, with significant variability in fiber quality, which falls far short of meeting the demands for high-end textile production worldwide [3, 4]. Consequently, improving the quality and yield of cashmere goats has become a critical challenge for the cashmere goat farming industry and the textile sector.

The growth of cashmere exhibits significant seasonality, characterized by alternating phases of cashmere growth and shedding, which are determined by the periodic activity of secondary hair follicles (SHFs). The growth cycle of SHFs consists of three phases: anagen (growth phase), catagen (regression phase), and telogen (resting phase) [5]. Studies have shown that the cyclic growth of hair follicles, as well as the proliferation and differentiation of associated cells, are synergistically regulated by hormonal factors (e.g., melatonin, prolactin, and thyroid hormones) and signaling pathways, including the Wnt/β-catenin, fibroblast growth factor (FGF), insulin-like growth factor (IGF), and transforming growth factor-β (TGF-β) pathways [6–8]. The growth of hair follicles has been extensively studied using both single-omics and multiomics approaches. For example, Ranran Zhao R et al. established the key gene regulatory network involved in inducing hair follicle development in Aohan fine wool sheep using transcriptomics [9]. Ma S C et al. applied metabolomics to reveal different feeding strategies that regulate hair follicle cycles in cashmere goats by influencing the expression of skin metabolites [10]. Furthermore, Wang X W et al. integrated transcriptomics and metabolomics to demonstrate that stress inhibits hair follicle growth by altering glucose and lipid metabolism pathways [11]. The above studies underscore the utility of multiomics technologies as powerful tools for elucidating the molecular mechanisms underlying skin hair follicle growth.

In recent years, numerous studies have demonstrated that melatonin (MT) promotes skin maturation and improves fleece and cashmere yields in fur-bearing animals. Our previous study revealed that melatonin implantation in adult cashmere goats prolonged the growth phase of secondary hair follicles, thereby increasing cashmere quality and yield. Another study revealed that melatonin enhanced the antioxidant capacity, inhibited apoptosis, and increased the number of SHFs [12, 13]. However, the pleiotropic effects of melatonin in promoting hair follicle growth (including rhythmic, antioxidant, and anti-inflammatory properties) present challenges in elucidating the roles by which melatonin regulates hair follicle development. Studies on the regulation of hair follicle growth by melatonin have focused primarily on genes and phenotypes, with limited attention given to whether melatonin affects metabolites in skin hair follicles and blood. Therefore, this study aimed to explore the effects of melatonin on gene expression, metabolic changes, gene–metabolite interactions, and metabolic signaling pathways associated with hair follicle development using integrated transcriptomic and metabolomic analyses. We expect to further elucidate the molecular mechanisms through which melatonin regulates the development of SHFs in cashmere goats, providing a theoretical foundation for its application in promoting cashmere production.

Materials and methods

Experimental design

Twenty-four female adult Inner Mongolia white cashmere goats, aged 2 years, with similar body weights (31.32 ± 3.55) and good physical condition, were selected and randomly divided into two groups, including the control group (CK) and the melatonin group (MT), with 12 goats in each group. The MT group received two melatonin implants (2 mg/kg, with a sustained-release period of 2 months) in the neck region in April (the onset of secondary follicle development) and again in June [12]. The control group received no treatment. All goats were grazed for 8 h daily under consistent conditions with an adequate water supply. Feeding and management strictly adhered to the regulations of the experimental base. All cashmere goats used in this experiment were sourced from the YiWei white cashmere goat farm in Ordos city, Inner Mongolia Autonomous Region, China.

Sample collection and detection

Since only a small amount of skin tissue was collected in this study, with minimal risk of significant bleeding, the cashmere goats did not undergo anesthesia or euthanasia. Skin and blood samples were collected 30 days after the first melatonin implantation. Four skin samples were obtained using a skin sampler (10 mm in diameter) from the left side of the scapular region of cashmere goats at the upper one-third of the line connecting the dorsal midline and the ventral midline. After collection, hemostasis was achieved by applying gauze pressure, followed by the application of Yunnan Baiya (Yunnan, China). The skin samples were thoroughly rinsed with phosphate-buffered saline (PBS), and two pieces were placed in freshly prepared 4% paraformaldehyde fixation solution for 24 h. The samples were subsequently dehydrated in 30% and 50% ethanol for 2 h each and preserved in 70%

ethanol to facilitate subsequent paraffin embedding and sectioning. The samples were used for observation of follicle structures and parameter measurements. The main parameters included primary follicle density (PFD), active secondary follicle density (ASFD), secondary follicle density (SFD), the ratio of active secondary follicles to primary follicles (active secondary follicle/primary follicle density, S*f*/P), and the ratio of secondary follicle density to primary follicle density (S: P). The other two skin samples were preserved in RNA stabilization solution for subsequent transcriptomic analysis, real-time quantitative polymerase chain reaction (q-PCR), and metabolomics measurements. Blood samples were collected between 22:00 and 23:00, centrifuged at 2500 × g, and preserved in liquid nitrogen for serum metabolomics analysis.

Cashmere samples were collected during the combing season of the following year. A 5 cm × 5 cm area was selected from the midsection on the left side of each cashmere goat's body. The coarse hair and cashmere were trimmed close to the skin and placed into numbered resealable bags for preservation. The cashmere diameter, length, and yield were measured following the methods described by Duan et al. [14].

RNA extraction, transcriptome data, and q-PCR

Total RNA was extracted from the skin samples (CK/MT = 6) using an RNA extraction kit (R1200, Solarbio, Beijing) according to the manufacturer's instructions. The integrity of the RNA was verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA) and agarose gel electrophoresis. The total RNA requirement for library construction was 1 μg, with a concentration ≥ 30 ng/μL, RQN > 6.5, and OD260/280 between 1.8 and 2.2. RNA purification, reverse transcription, library construction and sequencing were performed at Shanghai *Majorbio* Biopharm Biotechnology Co., Ltd., according to the manufacturer's instructions [15]. The raw paired-end reads were filtered using fastp (https://github.com/OpenGene/fastp) to obtain clean reads. The clean reads were subsequently separately aligned to the reference genome (Capra hircus, http://asia.ensembl.org/Capra_hircus/Info/Index) in orientation mode using HISAT2 (hierarchical indexing for spliced transcript alignment 2) software. The mapped reads of each sample were assembled using String-Tie with a reference-based approach. The functional annotation of the genes and transcripts was performed using multiple databases, including NR (nonredundant protein sequence database), SwissProt (Swiss protein), Pfam (protein family database), EggNOG (evolutionary genealogy of genes: nonsupervised orthologous groups), GO (gene ontology), and KEGG (Kyoto Encyclopedia of Genes and Genomes) databases. The expression levels of genes/transcripts were quantified using the expression quantification software RSEM (RNA-Seq by expectation-maximization) with TPM (transcripts per million) as the measurement unit. Differential gene expression analysis was performed using the DESeq2 (differential expression analysis based on the negative binomial distribution) software for both the CK group ($n = 6$) and the MT group ($n = 6$). The criteria for identifying differentially expressed genes (DEGs) were set as follows: $|\log2(\text{fold change})| \geq 1$ and P-adjust < 0.05.

The extracted RNA from the skin was then reverse-transcribed into cDNA via a cDNA reverse transcription kit (11203ES03, Xusheng, Shanghai). q-PCR was performed using ChamQ SYBR q-PCR Master Mix (Vazyme Biotech Co., Ltd., Nanjing, China) and assayed on a real-time PCR detection system (Roche, Switzerland). GAPDH was used as the reference gene to normalize expression levels, and the data were processed using the $2^{-\Delta\Delta CT}$ method. Information on the primer sequences is provided in Table S1.

Metabolite extraction and LC–MS analysis

A 100 mg skin sample was thawed and prepared for metabolite extraction. The detailed procedure is provided as follows [16]: (1) Skin samples were immersed in 120 μL of prechilled 50% methanol buffer (methanol: distilled water = 1:1), vortexed for 1 min, incubated at room temperature for 10 min, and stored overnight at -20 °C. (2) The mixture was centrifuged at 4000 × g for 20 min, and the supernatant was transferred to a 96-well plate for storage at -80 °C. (3) Metabolite detection was performed by Shanghai *Majorbio* Biotechnology Co., Ltd. The raw metabolite data were analyzed using the *Majorbio* cloud platform [17]. First, the metabolite expression data underwent preprocessing steps on the cloud platform software, including filtering, imputation, normalization, and logarithmic transformation, to minimize or eliminate errors introduced during the experimental and analytical processes. The metabolites in the preprocessed "Metab Table_Origin" dataset were subsequently annotated using the KEGG and HMDB databases. Statistical analyses were conducted using the R software package (Version 1.6.2) and Python (Version 1.0.0). The evaluation was conducted using MetaX software for principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). By default, differentially regulated metabolites (DRMs) were screened using a combination of univariate statistical analysis (t test), multivariate statistical analysis (OPLS-DA/PLS-DA), and fold change (FC) values. The default screening criteria were set as $P < 0.05$, VIP > 1, and FC < 1 or FC > 1 (FC filtering was disabled by default). The detection and analysis of serum metabolites followed the procedures outlined for skin metabolomics.

Joint analysis of metabolites and genes

To investigate the regulatory network of genes and metabolites involved in melatonin-mediated hair follicle development, we utilized the SciPy software package (Python) to perform an integrated annotation of all differentially expressed gene (DEG) sets and metabolite (DRM) sets in the KEGG database. We specifically focused on significantly coenriched pathways. Subsequently, Pearson correlation analysis was conducted to examine the relationships between the coannotated metabolites and genes.

Statistical analysis

The data were compiled and analyzed using EXCEL 2020, and hair follicle characteristics, wool fineness, length, and yield comparisons were conducted using the general linear model in SPSS 26.0. The results are expressed as means ± standard error (means ± SD). Transcriptomic and metabolomic data were analyzed and visualized using the *Majorbio* cloud platform (https://cloud.majorbio.com).

Results

Melatonin enhanced cashmere production performance

The characteristics of the cashmere are shown in Table 1. The fiber fineness of the MT group was 13.97 ± 0.98 μm, which was significantly lower than that of the control group (14.90 ± 0.50 μm, $P < 0.01$). The length in the control group (8.00 ± 1.00 cm) was significantly shorter than that in the MT group (9.41 ± 1.10 cm, $P < 0.01$). Additionally, the cashmere yield of the MT group was significantly increased 345.41 g compared with the control group.

Melatonin promotes the early growth of SHFs and increases the number of ASHFs and SHFs

Figure 1-A illustrates the morphological structure of hair follicles in the control and MT groups. The transverse sections of MT-1 revealed a significantly greater number of SHFs with red inner root sheaths than did those of CK-1, indicating a greater number of ASHFs in the MT group than in the control group. In longitudinal sections of MT-2, the roots of the SHFs were located farther from the skin surface than those of CK-2, suggesting that the developmental stage of hair follicle clusters in the MT group was more advanced than that in the control group. The characteristics of hair follicles in both the CK and MT groups are shown in Fig. 1-B. No significant difference in PFD was noted between the MT group (2.86 ± 0.14, n/mm²) and the CK group (2.82 ± 0.14, n/mm²) ($P > 0.05$). However, compared with the control group, the MT group exhibited greater SFD (22.72 ± 3.30 vs. 34.80 ± 3.39, n/mm²), S/P ratio (8.03 ± 1.42 vs. 12.61 ± 1.35), active secondary follicle density (12.61 ± 4.02 vs. 32.00 ± 3.80, n/mm²), and S*f*/P ratio (4.19 ± 1.79 vs. 11.57 ± 1.62), with highly significant differences ($P < 0.01$).

Table 1 Effects of melatonin on the quality and yield of cashmere

Items	CK	MT	*P* value
Diameter, μm	14.90 ± 0.52	13.97 ± 0.98	< 0.01
Length, cm	8.00 ± 1.00	9.41 ± 1.10	< 0.01
Cashmere yield, g	782.50 ± 54.07	1127.91 ± 224.75	0.05

Transcriptomic analysis of skin hair follicles under melatonin intervention

The transcriptomic data of the skin samples revealed 509 DEGs, including 235 upregulated and 274 downregulated genes (Table S2). The expression profiles of these DEGs were visualized using a volcano plot and heatmap (Fig. 2-A, B). Among the DEGs, the top 10 most abundantly expressed genes were *ND4*, *KRTAP3-1*, *KRT10*, *KRTAP7-1*, *KRTAP13-3*, *KRTAP11-1*, *KRT1*, *POLR2L*, *S100A16*, and *TPM1*. In addition to members of the KRT and KRTAP families, other genes, such as *SH3BGRL3*, *AQP3*, *VDR*, *LEF1*, *PPP1R3B*, *PTCH1/2*, *HSD11B1*, and *MMP1/27*, were also associated with skin and hair follicle development. A subset of hair follicle development-related genes was selected for q−PCR validation, and the results were consistent with the RNA-seq data (Fig. 3-A). KEGG pathway enrichment analysis revealed 23 significantly enriched pathways ($P \leq 0.05$, Table S3), with the top 10 pathways including basal cell carcinoma, pathways associated with cancer, *Staphylococcus aureus* infection, melanogenesis, breast cancer, complement and coagulation cascades, neuroactive ligand−receptor interactions, cell adhesion molecules, the Wnt signaling pathway, and the cell cycle (Fig. 3-B).

Metabolomic analysis of skin hair follicles under melatonin intervention

Nontargeted metabolomics analysis of the skin samples was performed using UHPLC-LS/MS (ultrahigh-performance liquid chromatography-light scattering/mass spectrometry), which identified 842 metabolites, including 564 in positive ion mode (pos) and 278 in negative ion mode (neg). These metabolites were annotated in the HMDB (human metabolome database), lipid maps, and KEGG databases (Table S4). The OPLS-DA (orthogonal partial least squares discriminant analysis) model yielded R^2 (explained variance) and Q^2 (predictive ability) values of 0.9671 and −0.028, respectively, indicating model stability and the absence of overfitting (Fig. S1). Differentially regulated metabolites (DRMs) were screened on the basis of variable importance (VIP) values (VIP > 1) from the OPLS-DA model and *P* values ($P < 0.05$) obtained from t tests. A total of 177 DRMs were identified, including 127 upregulated and 50 downregulated metabolites (Table S5). The volcano plot (Fig. 4-A) provides detailed

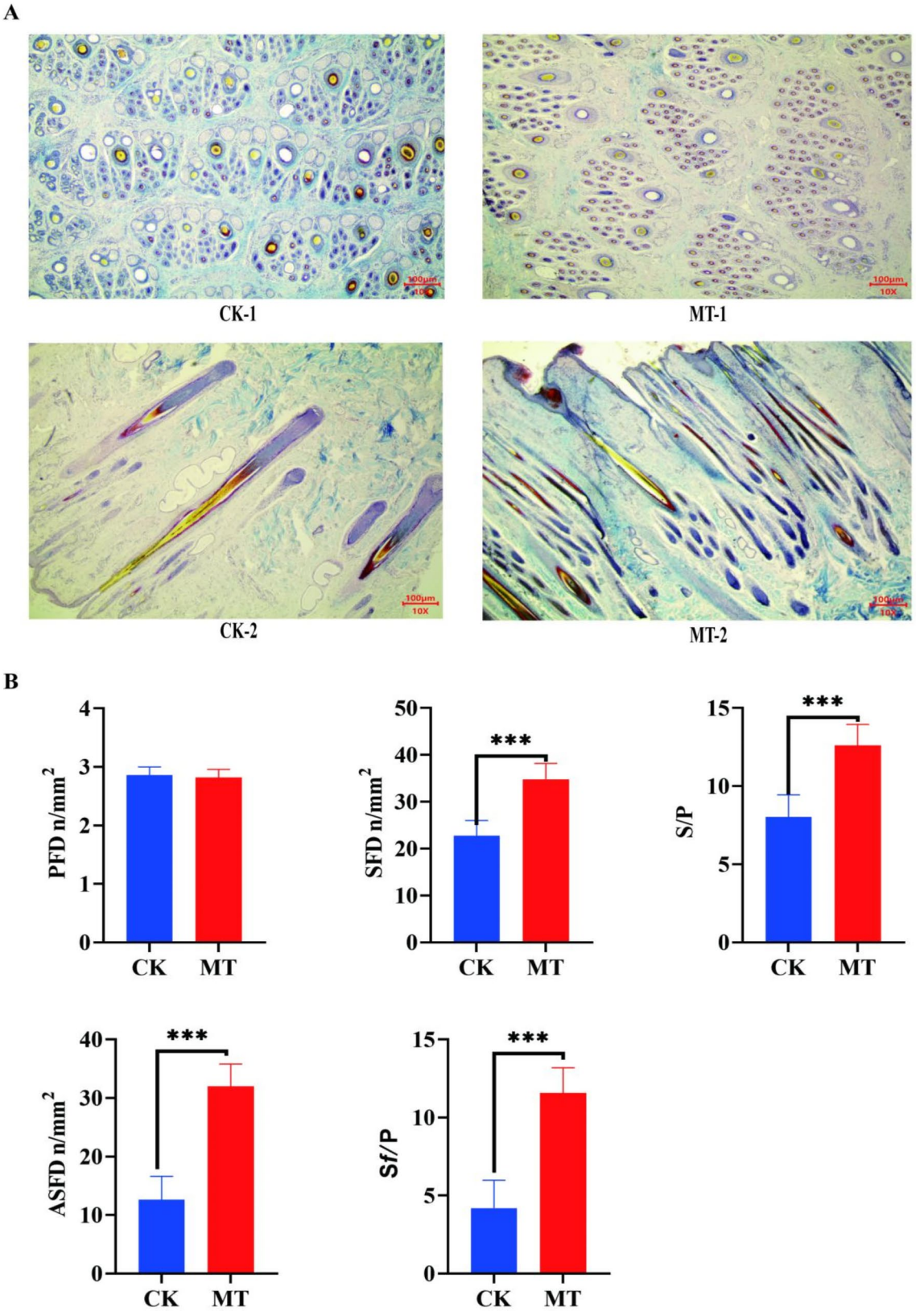

Fig. 1 Hair follicle morphology and parameters. (**A**) CK-1/MT-1 is the cross section of the CK/MT group (10X); CK-2/MT-2 is the longitudinal section of the CK/MT group (10X). (**B**) Primary follicle density, PFD; secondary follicle density, SFD; secondary follicle density/primary follicle density, S/P; active secondary follicle density, ASFD; active secondary follicle/primary follicle density, Sf:P. * $P < 0.05$, ** $P < 0.01$, CK vs. MT

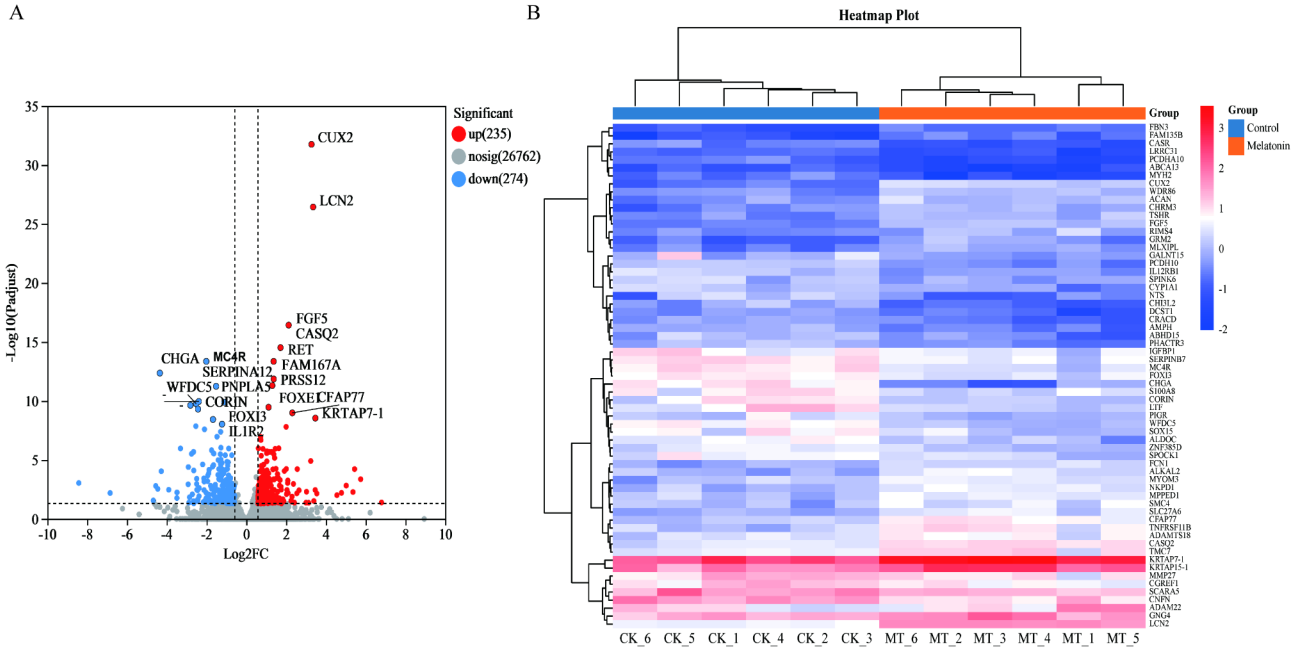

Fig. 2 mRNA expression in the skin and hair follicles of cashmere goats treated with melatonin (MT) or not treated (CK). (**A**) Volcano plots of DEGs. (**B**) Heatmaps of DEGs. The up- and downregulated genes are shown in red and blue, respectively

information on the DRMs and their changes. Metabolites potentially associated with skin hair follicles and their derivatives, such as l-carnitine, prostaglandin F1a, testosterone, propionylcarnitine, 8-oxoguanosine, and glutamate, are highlighted. The heatmap demonstrates the clustering of the top 20 DRMs, indicating the reliability of the data across all samples, with a greater proportion of downregulated metabolites (Fig. 4-B). DRMs were subsequently classified on the basis of the HMDB metabolite database, including lipids and lipid-like molecules (31.62%), organic acids and derivatives (24.26%), organoheterocyclic compounds (13.24%), and organic oxygen compounds (7.35%) (Fig. 4-C). KEGG pathway enrichment analysis of the DRMs revealed significant impacts on pathways such as alpha-linolenic acid metabolism; arginine and proline metabolism; glyoxylate and dicarboxylate metabolism; the citrate cycle (TCA cycle); arginine biosynthesis; alanine, aspartate and glutamate metabolism; GABAergic synapses; and lysine biosynthesis (Fig. 4-D, Table S6).

Metabolomic analysis of serum under melatonin intervention

Subcutaneous melatonin implantation directly affects hair follicle growth and may also exert indirect effects by permeating the bloodstream. Therefore, we performed a metabolomic analysis of blood samples from the MT and CK groups. A total of 1,162 metabolites were identified, including 714 in positive mode and 448 in negative mode (Table S7). Using the OPLS-DA model, 122 DRMs were identified, including 108 upregulated and 14 downregulated metabolites (Table S8, Fig. S2). A subset of these metabolites was annotated in the volcano plot (Fig. 5-A). A clustering heatmap of the top 20 DRMs revealed that the proportion of upregulated metabolites exceeded that of downregulated metabolites, contrary to the results observed in the skin (Fig. 5-B). The categories of differentially abundant metabolites in the serum and their relative proportions were similar to those identified in the skin (Fig. 5-C). KEGG pathway enrichment analysis revealed that these metabolites are predominantly associated with pathways such as protein digestion and absorption, central carbon metabolism in cancer, aminoacyl-tRNA biosynthesis, d-amino acid metabolism, ABC transporters, phenylalanine metabolism, mineral absorption, and cocaine addiction (Fig. 5-D, Table S9).

Integrative analyses of the transcriptome and metabolome

We conducted an integrated analysis of transcriptomic and metabolomic data to investigate potential relationships between secondary hair follicle development genes and metabolites under melatonin intervention. DEGs in the skin were annotated with metabolites from both the skin and blood using the KEGG database, which identified 53 and 52 signaling pathways (Fig. 6-A, B), respectively. Notably, the significantly enriched pathways in the skin included the FOXO signaling pathway, pyrimidine metabolism, linoleic acid metabolism, and arginine and proline metabolism (Fig. 6-C, Table S10). In the blood, the enriched pathways included long-term depression,

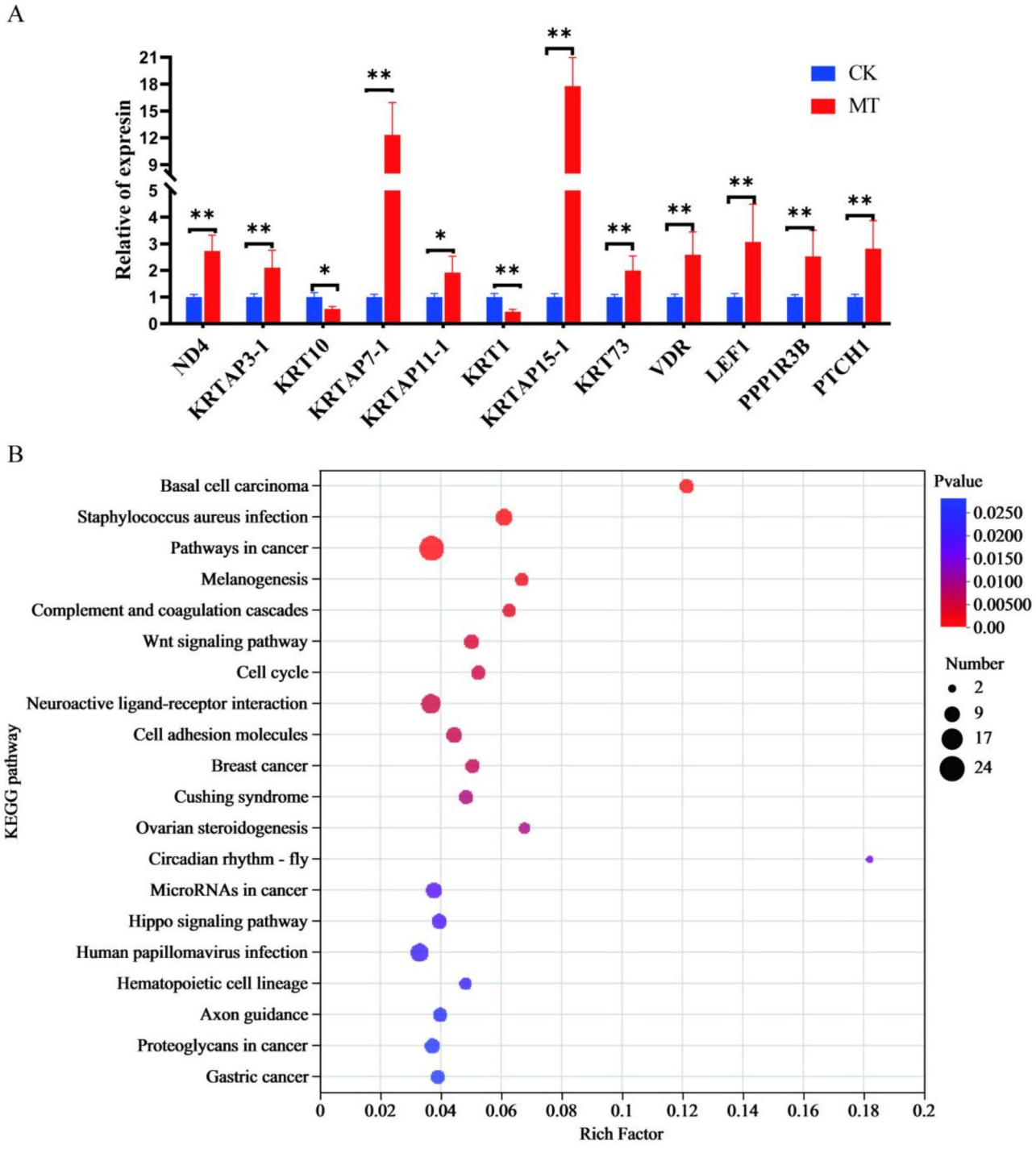

Fig. 3 mRNA expression in the skin of cashmere goats treated with melatonin (MT) or not treated (CK). (**A**) The expression levels of 12 DEGs determined via q–PCR. (**B**) Diagrams of the degree of KEGG pathway enrichment of DEGs in the skin and hair follicles

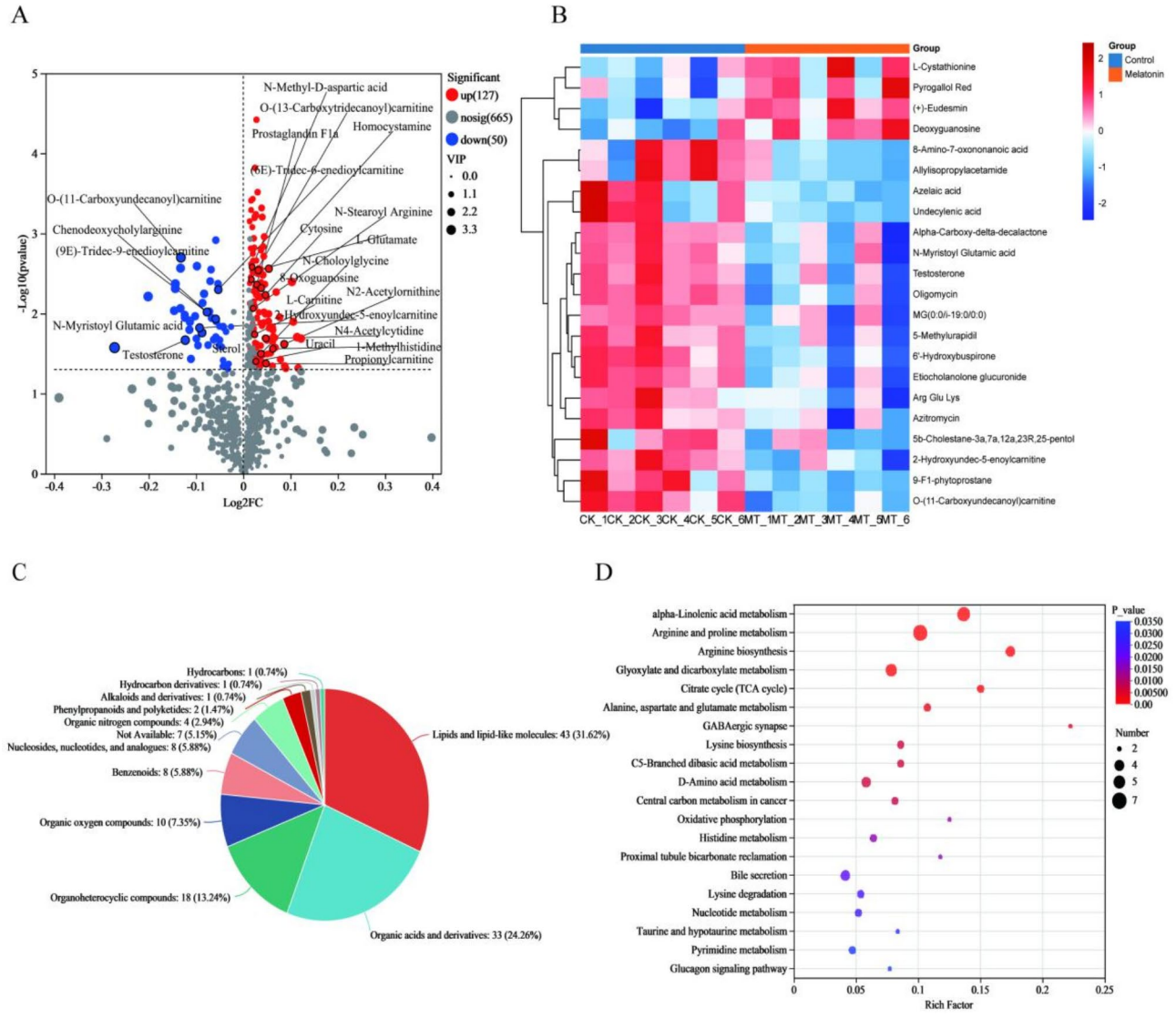

Fig. 4 Analysis of DRMs in skin. (**A**) Volcano diagram for VIP and P screening. (**B**) Heatmap of differentially abundant metabolites. (**C**) Identification of differentially abundant metabolites of skin from HMDB superclasses. (**D**) KEGG pathway of differentially abundant metabolites

melanogenesis, protein digestion and absorption, glutamatergic synapses, central carbon metabolism in cancer, the FOXO signaling pathway, cocaine addiction, neuroactive ligand–receptor interactions, and arginine and proline metabolism (Fig. 6-D, Table S11). Figure 6. E and F show the correlations between DEGs and DRMs within the KEGG coannotated pathways.

Discussion

MT is a highly multifunctional signaling molecule that acts on nearly all tissues, organs, and cells and has various biological functions, including sleep regulation, antioxidative effects, anti-inflammatory properties, and antiapoptotic activities [18, 19]. In the context of animal production, MT was first applied to fur-bearing animals such as mink, foxes, and rex rabbits to promote early fur maturation and increase fur yield [20–22]. Studies have demonstrated that melatonin promotes cashmere growth by increasing fiber length and reducing fiber diameter [23, 24]. Consistent with these findings, the present study also confirmed melatonin's effects on promoting cashmere growth. The quality and yield of cashmere are widely recognized to be influenced by the final number of active secondary follicles during anagen in the current growth cycle. Therefore, the direct mechanism by which melatonin promotes hair follicle growth is its ability to preserve more ASHFs [25, 26]. In experiments involving fur animals such as rabbits and chinchillas, melatonin improved the morphology and parameters of SHFs [27, 28]. Previous histological analyses of cashmere goat SHFs during the growth phase revealed that melatonin induced earlier initiation of secondary follicles and increased the

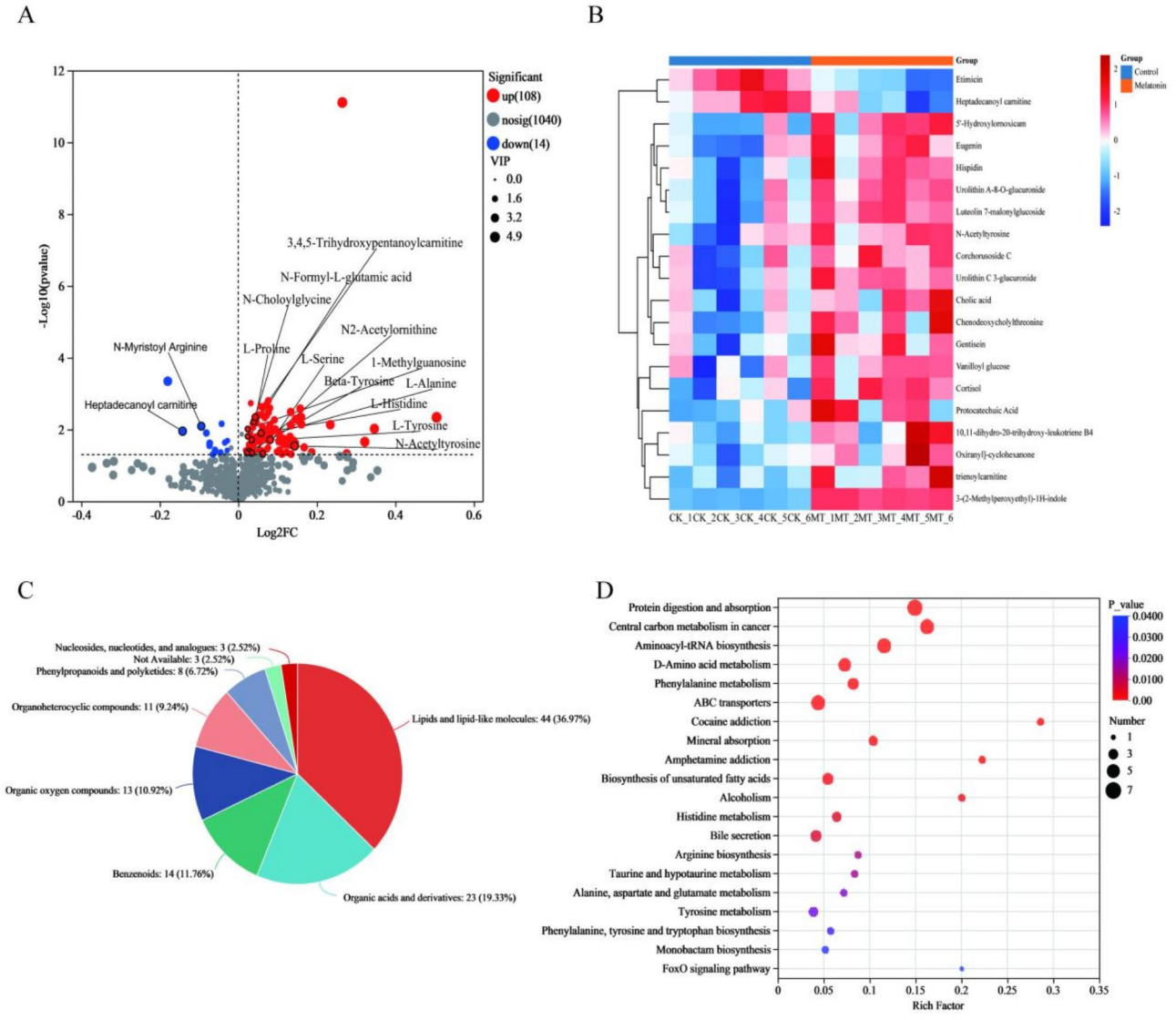

Fig. 5 Analysis of DRMs in serum. (**A**) Volcano diagram for VIP and P screening. (**B**) Heatmap of differentially abundant metabolites. (**C**) Identification of differentially abundant metabolites in serum from HMDB superclasses. (**D**) KEGG pathway of differentially abundant metabolites

number of active secondary follicles. In this study, one month after implantation, the SHF parameters and morphology in the MT-treated group were superior to those in the control group, which is similar to earlier findings [29, 30].

KRT and *KRTAP* are the primary components of cashmere fibers and are closely related to cashmere fiber morphology and hair follicle curvature. *KRT* proteins play crucial roles in maintaining the integrity and stability of hair follicle cell structures and regulating the hair follicle cycle [31]. Several studies have shown that endocrine hormones such as estrogen and melatonin regulate the expression of certain KRTs, thereby influencing hair follicle development and increasing cashmere yield [32–34]. In this study, we identified eight significantly differentially expressed KRTs, among which *KRT1*, *KRT10*, *KRT24*, and *KRT77* were downregulated, whereas *KRT8*, *KRT28*, *KRT72*, and *KRT73* were upregulated. Interestingly, the expression of the screened *KRTAP* family genes, including *KRTAP3-1*, *KRTAP7-1*, *KRTAP11-1*, *KRTAP13-3*, and *KRTAP15-1*, tended to increase. Both *KRTAP* polymorphisms and expression levels influence the structure of wool fibers. For example, *KRTAP6*, *KRTAP7*, and *KRTAP8* exhibit significant variability in wool fibers and are strongly associated with wool fiber diameter [35, 36]. However, studies on the molecular mechanisms by which melatonin regulates *KRT*s and *KRTAP*s to influence the fineness and length of cashmere fibers are limited. KEGG enrichment analysis results revealed several cancer-related signaling pathways in which the expression of key genes affected by melatonin. Previous research has shown that melatonin plays a role in cancer and tumor

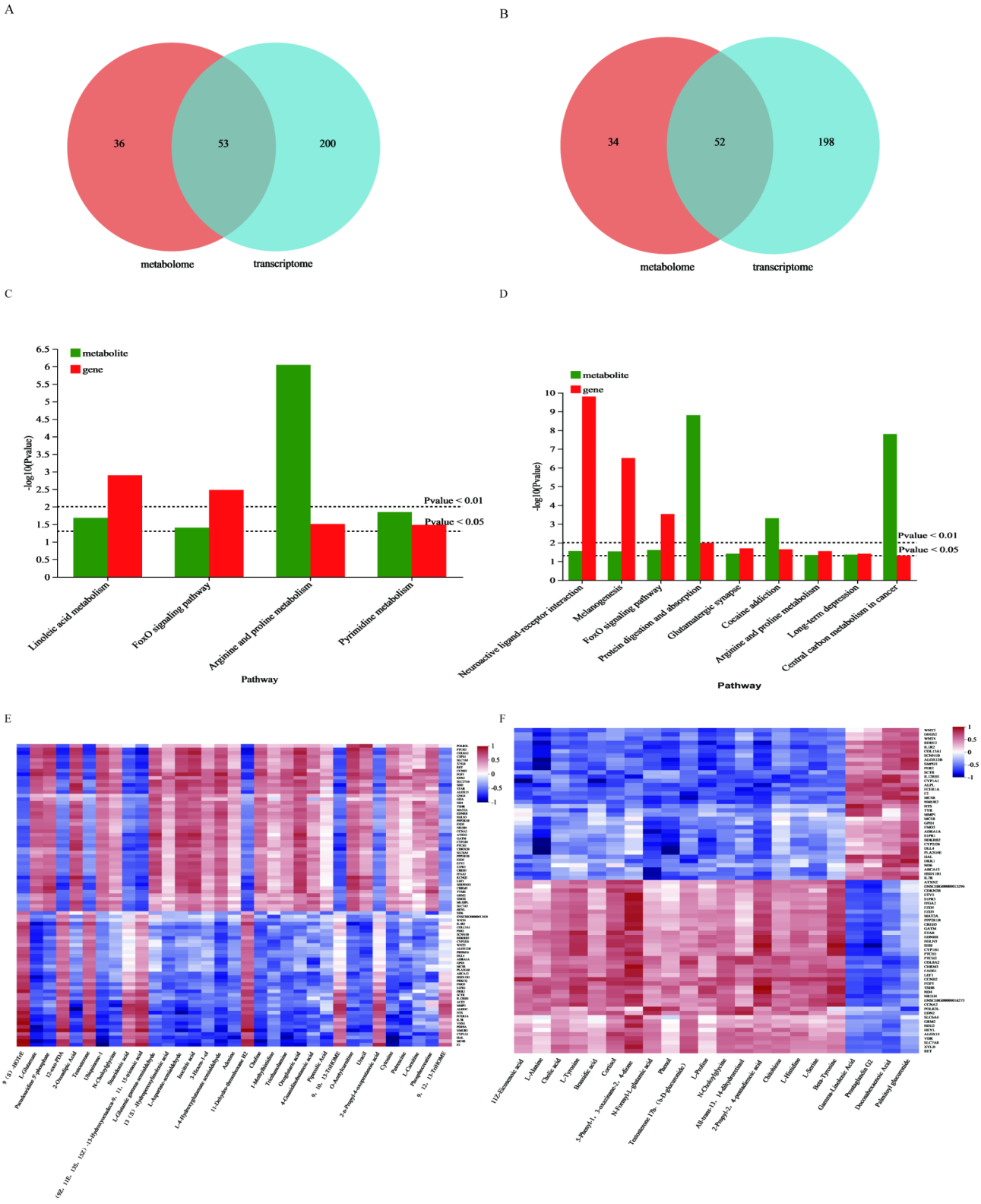

Fig. 6 Integration analysis of DEGs and DRMs in skin and serum. (**A**) and (**B**) Venn diagram of the KEGG pathway annotations for skin and serum samples. (**C**) and (**D**) Statistics of significant coenriched pathways in skin and serum. (**E**) and (**F**) Correlation analysis plot of the differential metabolome and transcriptome in skin and serum

therapy, primarily by increasing the sensitivity of cancer cells to treatment and promoting apoptosis through the modulation of apoptotic mediators [37]. Moreover, we observed significant changes in key genes of the Wnt signaling pathway, including *LEF1*, *WNT3/4*, and *FZD3/5*. The Wnt signaling pathway is a classical pathway that regulates hair follicle growth and development. Notably, melatonin stimulates Wnt signaling in hair follicle cells and regulates hair follicle stem cells to promote cashmere growth [38].

Hair follicles are appendages of the skin, and their growth and development are closely related to skin tissues and blood circulation. The skin provides structural support and protective functions to hair follicles, whereas blood supplies essential nutrients. Furthermore, metabolites and their derivatives in skin tissues and blood affect hair follicle development and cyclic growth [10, 39]. Studies on melatonin-regulated metabolic pathways have focused predominantly on plants, with few reports in fur-bearing animals. Therefore, this study performed untargeted metabolite profiling on skin follicle and blood samples. The identified differentially abundant metabolites included mainly lipids and lipid-like molecules, organic acids and derivatives, aromatic compounds, oxygen-containing organic compounds, and nucleotides and analogs, among which lipids accounted for a substantial proportion. Lipid metabolites play indispensable roles in animal physiology and biochemistry. Andrieu-abadie N et al. and Vescovo G et al. reported that L-carnitine inhibits cardiomyocyte apoptosis and prevents heart failure [40, 41]. Derivatives of carnitine, such as l-carnitine-l-tartrate and propionyl-l-carnitine, significantly promote hair follicle development and effectively treat hair loss [42, 43]. In this study, we observed significant upregulation of l-carnitine, o-acetylcarnitine, propionylcarnitine, and o-(13-carboxytridecanoyl)carnitine in the skin of the melatonin-treated group as well as certain derivatives found in the blood. In addition, prostaglandin f1a and testosterone in the skin are significantly upregulated and downregulated, respectively, and prostaglandin f1/2α and its analogs can reverse androgen-induced hair loss by promoting hair follicle growth [44]. As previously mentioned, keratin is a significant component of the skin, hair follicles, and wool, and its basic units include peptides and amino acids. Supplementing melatonin in the late stages of pregnancy in cattle improves maternal–fetal amino acid circulation, influencing fetal development. Moreover, melatonin supplementation increased glutamate and Cys levels and alleviated inflammation in a mouse enteritis model [45, 46]. In the present study, l-glutamate, leucyl-leucine, and prolylproline were significantly upregulated in the skin metabolism data, and l-tyrosine, l-histidine, l-serine, l-alanine, l-proline, and n-acetyltyrosine levels were upregulated in the blood. Furthermore, multiple amino acid metabolism-related pathways were significantly enriched in the KEGG signaling pathways for both the skin and blood. Based on these findings, we hypothesize that melatonin promotes secondary hair follicle development by modulating amino acid metabolism. Nucleotides and their metabolites determine the sequence of amino acids and influence energy metabolism and material transport. In our study, significant upregulation of nucleotide metabolites was observed in both the skin and blood, suggesting that melatonin accelerates hair follicle metabolism in the skin.

Metabolites, as the ultimate product of gene expression, are crucial links between genotype and phenotype. Integrating transcriptomic and metabolomic analyses enables a more comprehensive investigation of the molecular processes underlying gene–metabolite networks. Combined omics analysis of skin and blood revealed enrichment of several signaling pathways, among which the FOXO-signaling pathway and the arginine and proline metabolism pathway were significantly enriched in both tissues in this study. The FOXO signaling pathway regulates various physiological and pathological processes, including cell proliferation, apoptosis, metabolism, and aging [47]. The arginine and proline metabolism pathway has been shown to influence both skin wound healing and skin fibrosis; moreover, a study indicated that this pathway was significantly enriched in multiomics analyses of cashmere goats with varying coat types, suggesting a potential link between the arginine and proline metabolism pathways and fiber length in cashmere goats [48, 49]. Correlation analyses between metabolites and genes in this study revealed strong associations between several DEGs and DRMs. We hypothesize that melatonin plays a regulatory role in gene–metabolite interactions. However, this regulatory molecular mechanism requires further investigation and validation.

Conclusion

This study analyzed transcriptomics and metabolomics in skin and blood samples following melatonin intervention, and a substantial number of DEGs and DRMs were identified. On the basis of these findings, we demonstrated that melatonin promotes cashmere growth by regulating the expression of key genes involved in hair follicle development-related signaling pathways and metabolites involved in metabolic pathways. In summary, these results provide new insights and perspectives for further elucidating the biological mechanisms underlying the promotion of secondary hair follicle development in cashmere goats by melatonin.

Abbreviations

MT	Melatonin
SHFs	Secondary hair follicles
PFD	Primary follicle density

ASFD	Active secondary follicle density
SFD	Secondary follicle density
S*f*/P	Ratio of mature secondary follicles to primary follicles
S:P	Ratio of secondary follicle density to primary follicle density
q-PCR	Real-time quantitative polymerase chain reaction
VIP	Variable importance in projection
DEGs	Differentially expressed genes
DRMs	Differentially regulated metabolites
GO	Gene ontology
KEGG	Kyoto encyclopedia of genes and genomes
LC–MS/MS	Liquid chromatography–tandem mass spectrometry

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions

X.G.D.: investigation, data curation, software, writing—original draft; J.X.Q.: data curation, software; C.X.D.: software; L.W.H.: formal analysis; W.Z.: conceptualization, methodology, supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data availability

The datasets generated during the current study are available in the NCBI repository under the accession number ID PRJNA1208202.

Declarations

Ethics approval and consent to participate

The study design was reviewed and approved by the Animal Care and Use Committee of China Agricultural University (Beijing, China). (Approval No. AW71103202-1-1).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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