Hou *et al. BMC Genomics* (2024) 25:1070
https://doi.org/10.1186/s12864-024-10995-8

BMC Genomics

RESEARCH Open Access

Comparative ultrastructural and transcriptomic profile analysis of skin tissues from indigenous, improved meat, and dairy goat breeds

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Abstract

Background High-quality goatskins are valuable byproducts usually produced by indigenous goat breeds with poorer production performance in Asia and Africa. However, the genetic and molecular mechanisms underpinning goatskin's biomechanical properties (e.g., tensile strength) remain elusive. Mechanistic exploration of these traits could greatly aid the genetic improvement and genetic resource conservation of native breeds in these regions. To fulfill this purpose, we collected skin tissues from three goat breeds: Huai goat (HG), a Chinese native variety producing high-quality goatskins; Yudong meat goat (YDMG), a crossbreed of HG and Boer goat; Henan dairy goat (HNDG), a dairy goat breed.

Results Scanning electronic microscopy analysis of skin tissues found that the collagen fiber diameters, collagen fibril diameters, and crimps significantly differed among the three goat breeds; however, collagen fibril diameters are similar in HG and HNDG. A sum of 230, 775, and 86 differentially expressed genes (DEGs) were identified from YDMG versus HNDG, HG versus HNDG, and YDMG versus HG, respectively. Functional enrichment analysis suggested that signaling pathways involved in fatty acid, retinol, steroid metabolisms, and GO items related to the physical properties of the skin (e.g., collagen-containing extracellular matrix) are significantly overrepresented in DEGs identified from meat versus dairy goats. Furthermore, 106 DEGs (e.g., *COL1A1*, *COL1A2*, and *SPARC*) showed specific expression patterns in HG and YDMG versus HNDG. Items about biophysical features of skin (e.g., extracellular matrix organization and ECM proteoglycans) are markedly enriched. Protein-protein interaction analysis suggested that two growth factors (IGF1 and PDGFD) are latent collagen and other ECM protein expression modulators.

Conclusion Ultrastructural analysis of goat skin tissues suggested that collagen fibril diameter is not a major factor affecting goatskin quality. Transcriptomic profiles unveiled core genes and associated biological processes potentially

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involved in regulating goatskin quality. These discoveries shined new light on deeper understanding the mechanisms of hide-related traits in goat and other livestock.

Keywords Goatskin quality, Biomechanical property, Collagen fibril diameter, ECM organization, IGF1, Hormones

Background

Goatskins or goat leathers are important but often neglected byproducts with noticeable economic values in goat production in undeveloped countries in Asia and Africa [1]. Past studies revealed that breed is the crucial factor that determines the biomechanical properties of goat hides [2–4]. For instance, Yusuff et al. examined the biophysical characteristics of goatskins from pure and crossbred Nigerian goats, and found that several parameters, such as percentage elongation and cracking load, are significantly different among Red Sokoto, West African Dwarf, and their crossbreeds [5]. Similar phenomena occurred in other leather-producing species, including sheep [6], cattle [7], and rabbit [8]. Additionally, a recent study demonstrated that several traits (e.g., tensile strength) related to biophysical characteristic of sheepskins are moderate to highly heritable (h^2=0.28–0.82). However, associated genetic variations and underlying molecular mechanisms affecting biomechanical properties of leathers in livestock are less explored yet.

It is generally recognized that the biomechanical traits of leathers are shaped by skin dermis architecture, including diameter and orientation of collagen fibril [9], and the compactness of collagen fiber bundle interweaving [10]. Whereas, above factors contribute differentially to the biophysical properties of hides. For example, a moderate correlation exists between fibril diameter and hide tensile strength in bovine, but not sheep [11]. Although earlier literatures have affirmed that livestock variety significantly affects the biophysical features of leather, widespread comparative analysis of parameters pertaining to these factors among animal breeds are still lacking, especially in goats.

In addition to collagen and collagen fibers, skin dermis contains other building blocks, including elastin fibers and proteoglycans (PGs) [12]. These structural units are assembled into a complex extracellular matrix (ECM), which imparts mechanical strength of skin tissues [13]. Alterations of parameters related to skin mechanical features under physiological and pathological conditions are usually accompanied with changes of ECM organization. In aging human skins with reduced mechanical strength and resiliency, the expression abundances of genes encoding collagens, elastin, and GAGs were significantly downregulated [14]. Stimulated expressions of these genes via administrations of hormones, growth factors, and other agents are valid avenues to restore mechanical properties of human skins and anti-aging [15–17]. In mice, genetic mutations of *COL3A1* and a proteoglycan encoding gene-*SPARC*- lead to reduced collagen fibril diameter and decreased skin tensile strength [18, 19]. Furthermore, decreases of collagens content and the compactness of collagen fibers are associated with ultraviolet irradiation caused impairment of skin mechanical features in mice [20, 21]. However, most of these studies are mainly performed on humans and mice; little was known about such relationship on livestock.

In China, indigenous goats are generally dual-purpose breeds with disadvantage in production performance (e.g., smaller body conformation and lower growth rate), but yield hides with excellent quality. In past decades, widespread crossbreeding these native breeds with exotic meat (Boer) or dairy goat (Saanen) breeds has been proven as a successful strategy to enhance their production performance [22]. Although previous report demonstrated that the biomechanical parameters of goatskins from the first generations of hybrids are similar as local breeds [23], the outcomes of long-term hybridization are underexplored. In addition, unplanned crossbreeding resulted in drastic quantity decline of local breeds and nearly extinctions of certain breeds. For example, Huai goat (HG) is the goat variety well-known for yielding leather with a worldwide reputation called HuaiPi. Whereas, the amount of pure HG breeds dropped quickly in the last twenty years, and reports on the exploration of leather traits are still scarce.

In-depth investigations of the factors and molecular mechanisms influencing the biomechanical properties of goatskin are not only beneficial for genetic resource conservation of local breeds, but also are meaningful for genetic improvement. In the present study, we performed a preliminary ultrastructural and transcriptomic profile analysis of skin tissues from HG, a dairy goat breed (Henan dairy goat, HNDG), and a crossbreed of HG and Boer goat (Yudong meat goat, YDMG). Obtained data suggested that ECM organization rather than collagen fibril diameter might contribute significantly to goatskin quality. Furthermore, fatty acids, retinol, steroids, and growth factors play important roles in regulating collagen and other ECM protein expression in goat skin tissues.

Materials and methods

Skin sample collection and preservation

We collected goat skin samples from three goat breeds: Yudong meat goat (YDMG), Huai goat (HG), and Henan dairy goat (HNDG). Farm location and animal information were listed as follows: YDMG (Yudong Muye Co., Ltd, Ningling County, Shangqiu, China; ~ 1-year-old

males), HG (Yiming Huai goat breeding and improvement center, Shenqiu County, Zhoukou, China; ~ 1-year-old males), and HNDG (Mazhai Village, Xingyang City, Zhengzhou, China; ~2-year-old females). Brief breed characterization is provided in Table 1. Three goats of each breed with healthy conditions were chosen for image recordation and skin sample collection. Representative individual and hair images were taken using a P50E cellphone (Huawei Technologies Co., Ltd, Shenzhen, Guangdong, China). Then, hairs on the lateral backside of goats were clipped using a manual shaver, and skin tissues were sterilized via administration of iodine tincture and 75% alcohol. Subsequently, animals were locally anesthetized via subcutaneous injection of 0.3 mL hydrochloride procaine (Jilin Huamu Animal Health Products. CO., LTD., Changchun, China), and skin samples were harvested. Finally, skin wounds were surgically sutured and sterilized again. Samples were cut into two parts: one part was stored 2 h in fixation solution (G1102, Servicebio, Wuhan, China) at room temperature (RT) for scanning electronic microscopy (SEM), and then transferred into 4 ℃ for further storage and transportation; the other one was stored in liquid nitrogen for transcriptome sequencing and validation. All animal experimental procedures were supervised and approved by the Animal Welfare and Ethics Committee of Henan Agricultural University (approval number: HENAU-2018-039).

Scanning electronic microscopy (SEM)

Skin tissues (three replicates for each variety) were blocked with 1% OsO4 in 0.1 M phosphate buffer solution (PBS, pH=7.4) for 1.5 h at RT. Then, tissues were rinsed in PBS three times (15 min each), and gradually dehydrated in a series of ethanol (30%, 50%, 70%, 80%, 90%, 95%, and 100% twice; 15 min each) and isoamyl acetate solutions (10003128, Sinaopharm Group Chemical Reagent Company) for 15 min. Subsequently, skin samples were dried using a critical point dryer (K850, Quorum). After that, samples were attached to metallic stubs using carbon stickers and sputter-coated with gold for the 30s using a Lon sputtering instrument (MC1000, Hitachi). Finally, tissue images were captured using a scanning electron microscope (SU8100, Hitachi) by the Wuhan Servicebio Technology Company. ImageJ software (https://imagej.nih.gov/ij/index.html) was adopted to analyze SEM images for determining collagen bundle diameter, collagen diameter, and crimp. For each sample, at least 10 collagen bundles and collagen fibrils were selected for analysis.

Table 1 Description of goat breeds used in present study

Goat breeds	Production direction	Description
Huai goat (HG)	Meat and goatskin	A local goat breed with excellent leather quality and high fecundity, but inferior growth performance
Yudong meat goat (YDMG)	Meat	A crossbreed of Boer goat with HG
Henan dairy goat (HNDG)	Milk	A dairy goat breed similar as Saanen dairy goat

Total RNA extraction, RNA-seq library construction, and sequencing

Transcriptome sequencing was performed by Wuhan Yingzi Gene Technology Co., Ltd (https://www.yingzigene.com). Briefly, total RNA from all skin tissues (three replicates for each breed) was extracted using the RNAprep Pure Kit (DP432, TIANGEN Biotech Co., Ltd., Beijing, China) following the manufacturer's instructions. Then, the concentration and integrity of extracted RNA were determined using Qubit 4.0 (Thermo Fisher, Waltham, MA, USA) and Qsep400 (Bioptic, Taiwan, China) instruments. Subsequently, a total of 3 μg RNA was used to construct RNA libraries with the MGIEasy mRNA Library Prep Kit (MGI Tech Co., Ltd., Shenzhen, China). All procedures including polyA-selected RNA extraction, RNA fragmentation, random hexamer primed reverse transcription, and 150 nt paired-end sequencing by DNBSEQ-T7 (MGI Tech Co., Ltd., Shenzhen, China) were performed according to the user manual of the toolkit. Obtained raw data were used for downstream quality control and bioinformatic analysis.

RNA-Seq data analysis

Raw sequencing data were first filtered by fastp (0.23.2) [24], low-quality reads were discarded, and the reads contaminated with adaptor sequences were trimmed. Resultant clean reads were mapped to the goat reference genome from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_001704415.2/) using hisat2 (v2.2.1) [25]. Reads mapped to the exonic regions of each gene were counted by featureCounts (v2.0.3) [26], and then expression levels of genes were calculated by fragments per kilobase of exon model per million mapped fragments (FPKM). Differentially expressed genes between groups were identified using the DESeq2 package (v1.26.0) [27]. Adjusted p-value < 0.05 and $|\log_2(\text{fold change, FC})| \geq 1$ were adopted as the statistical significance criteria of differentially expressed genes (DEGs) between groups.

Gene ontology (GO), Kyoto encyclopedia of genes and genomes (KEGG), and reactome analysis

Before functional enrichment analysis of DEGs, gene ID was translated from goat to human using the online tool g: Orth (https://biit.cs.ut.ee/gprofiler/orth) [28]. Then, functional enrichment analysis (GO, KEGG, and Reactome) of DEGs or selected genes was performed through R package clusterProfiler 4.8.3 [29]. Related items with

adjusted p-value < 0.05 were thought of as significantly enriched. Data visualization was carried out using the R package ggplot2 [30].

Protein-protein interaction (PPI) analysis

Prediction of the protein-protein interaction relationship was performed using the online tool STRING (https://string-db.org/) [31]. The threshold of the combined score of PPI was set as 0.4. The PPI interaction network was visualized using Cytoscape 3.10.0 (https://cytoscape.org/) [32].

Quantitative real-time PCR (qRT-PCR)

Remaining total RNA extracted for transcriptomic sequencing were used for synthesis of cDNA. Briefly, reverse transcription reactions were performed using the cDNA synthesis kit (D7168S, Beyotime, Beijing, China). Quantitative real-time PCR analysis was performed in triplicates with Bio-Rad IQ5 Real-Time PCR system using BeyoFast™ SYBR Green qPCR Mix (D7601S, Beyotime, Beijing, China) according to the manufacturer's protocol. Relative levels of gene mRNA expression were determined using $2^{-\Delta\Delta Ct}$ method. *GAPDH* was set as the internal reference in all experiments. Selected genes and their primers information were detailed in Table S1.

Statistical analysis

Data in the present study were presented as Mean ± standard deviation (SD). Student's t-test was chosen to examine the mean differences between the two groups. The standard for statistical significance was $p < 0.05$, and statistical extreme significance was $p < 0.01$.

Results

Visual examination of coat composition and ultrastructural analysis of skin tissues from three goat breeds

To screen candidate functional genes and explore potential genetic molecular mechanisms underlying prominent goatskin quality of HG, we first visually examined the coat composition of HG, YDMG, and HNDG to minimize the possibility that skin transcriptomic differences are caused by coat composition. As displayed in Fig. 1A, the pelages of either meat goat breeds (YDMG and HG) or dairy goat breeds (HNDG) are mainly constituted of long, straight, and coarse guard hairs with white color. Next, we utilized SEM to characterize the ultrastructure of goatskin dermis, associated collagen fiber bundles and fibrils (Fig. 1B). Obtained results indicated that the collagen fiber diameter of HNDG is significantly thicker than those of YDMG and HG (t-test, $p < 0.01$; Fig. 1C). Meanwhile, the thickness of collagen fiber in HG is greater than YDMG (t-test, $p < 0.05$; Fig. 1C). Collagen fibril diameters are thicker in HG and HNDG than YDMG tissues ($p < 0.01$; Fig. 1D). No statistical significance was found between HG and YDMG. However, the width of collagen fibril crimp in HNDG skins is greater than that in HG and YDMG tissues ($p < 0.01$). Otherwise, collagen fibril crimp is wider in HG samples than YDMG ($p < 0.01$; Fig. 1E). Collectively, these results demonstrated that the three goat breeds have a similar coat composition, but distinct morphological parameters of skin dermis.

Identification of differentially expressed genes (DEGs) between goat breeds

To identify candidate functional genes about goatskin quality, we performed genome-wide RNA-sequencing (RNA-seq) to detect DEGs among three goat breeds. A total of 44,334, 560 ~ 72,770,682 raw reads were acquired from nine sequencing libraries. After the stringent quality control procedure, 6.29 ~ 10.77 G clean bases with qualified Q20 and Q30 scores were obtained. Subsequently, clean reads were mapped to the goat reference genome (No.: ARS 1.2), and the mapping ratio exceeds 93.58% in all samples. Data summary and detailed reads mapping information were provided in Table S2.

Next, we evaluated the global gene expression patterns of all samples using FPKM values. As displayed by the violin plot in Fig. 2A, all skin transcriptomes are highly similar at the global level. Pearson's correlation analysis indicated that the correlation coefficient among all samples ranges from 0.37 to 1.00, suggesting a large set of genes with divergent expression modes exist in tissues (Fig. 2B). Above finding is highly consistent with principal component analysis (PCA) result, in which all dots situate at separative regions on the plot (Fig. 2C). Detailed information of genes expressions is provided in Table S3.

Finally, we identified the DEGs among three goat breeds using the following criteria: $|\log2(\text{fold change})| \geq 1$ and adjusted p-value < 0.05. A total of 230 DEGs (84 upregulated and 146 downregulated) were found in HNDG compared with YDMG (Fig. 2D, E). Top-ranked DEGs (assessed by fold change) include *LOC102182746*,

GABRA3, *CHI3L2*, *CYP1A1*, *S100A8*, *LOC108634620*, *LOC108634550*, *LOC108634721*, and other genes. Compared with HG, 164 upregulated DEGs and 611 downregulated DEGs were identified in HNDG (Fig. 2F, G). Certain genes, including *LOC108634721* and *LOC108634619*, are specifically expressed in HG. In addition, 86 DEGs were discovered between YDMG and HG (Fig. 2H, I). Detailed information on DEGs identified from three groups is shown in Table S3.

Functional enrichment analysis of DEGs identified between meat and dairy goat breeds

We next performed KEGG and GO functional enrichment analysis of filtered DEGs. Results indicated that 29 KEGG signaling pathways were significantly

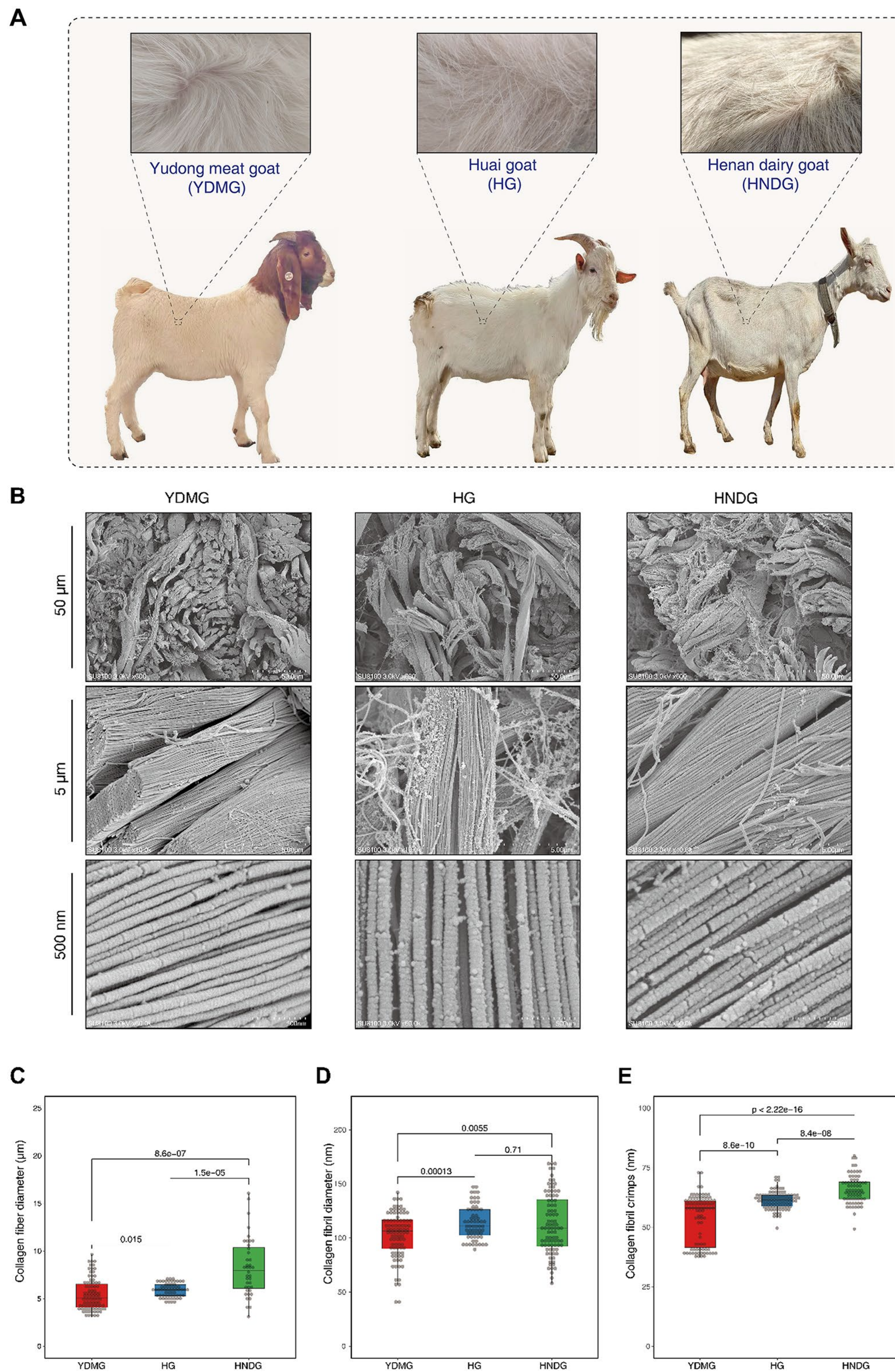

Fig. 1 Ultrastructural analysis of goat skin tissues using Scanning electronic microscopy. **A** Representative photo of three goat breeds and their furs. **B** Scanning electronic microscopy (SEM) image of goat skin tissues at three resolutions (50 µm, 5 µm and 500 nm). **C-E** Statistics of collagen fiber diameter, collagen fibril diameter, and crimps. Paired t-test was used to examine the difference between groups

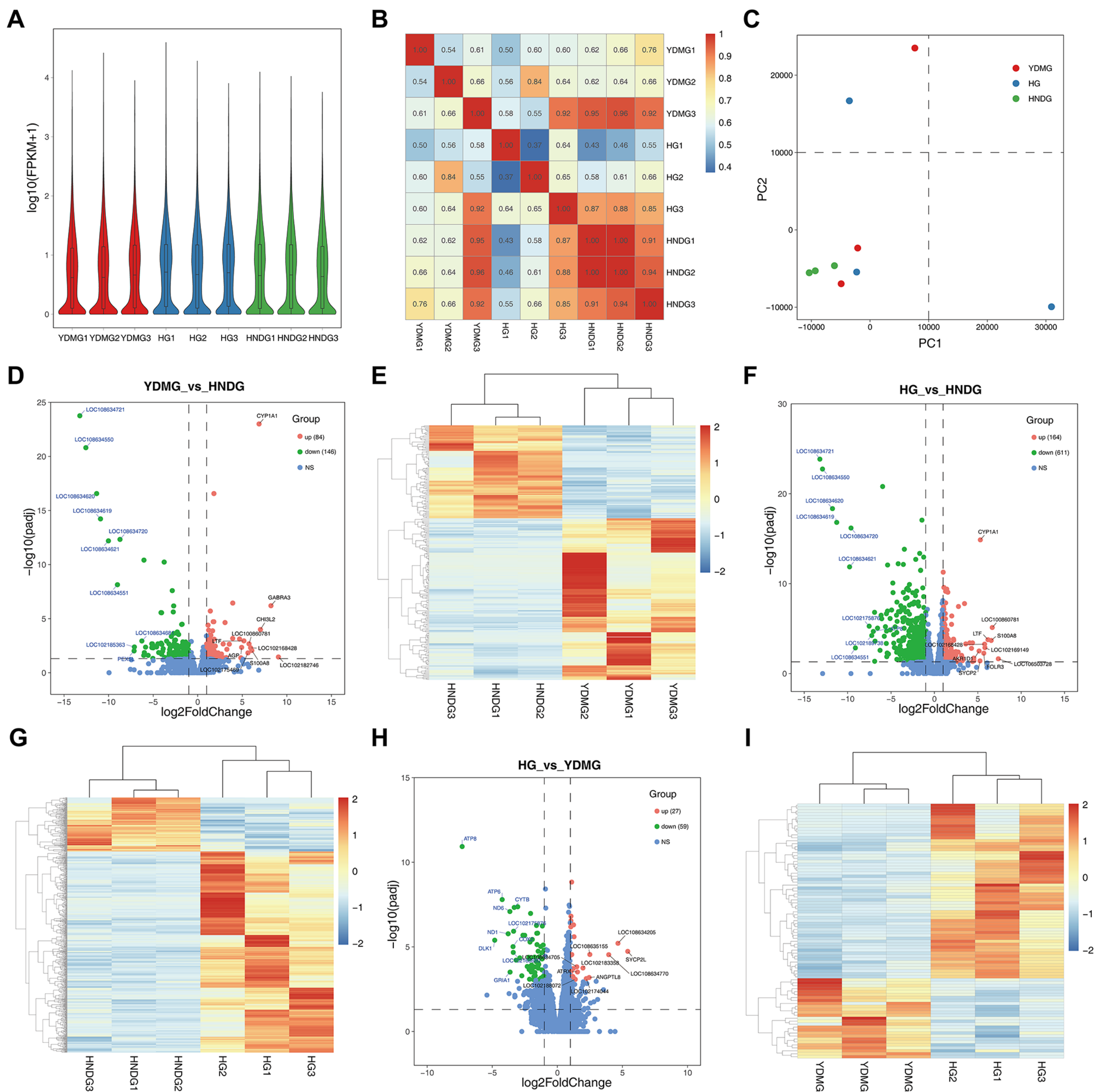

Fig. 2 Transcriptome analysis of skin tissues among three goat breeds. **A** Global gene expression pattern assessment using fragments per kilobase of exon model per million mapped fragments (FPKM). **B** Pearson correlation analysis of gene expression among sample. **C** Principal component analysis (PCA) of skin samples. **D-I** Volcano plot and heatmap of genes and differentially expressed genes (DEGs) among three groups, respectively

overrepresented for DEGs found between HG and HNDG. The top 20 enriched entries include protein digestion and absorption, ECM-receptor interaction, several lipid metabolism-related pathways (PPAR signaling pathway, fatty acid metabolism, fatty acid biosynthesis, and others), steroid hormone biosynthesis, retinol metabolism, and others (Fig. 3A). Of note, a group of collagen-encoding genes (e.g., *COL1A1*, *COL1A2*, and *COL3A1*), *ELN*, and growth factors-encoding genes (e.g., *IGF1*, *VEGFA*, and *PDGFD*) are associated with certain pathways, including protein digestion and absorption, ECM-receptor interaction, and PI3K-Akt signaling pathway (Fig. 3B). Moreover, retinol metabolism, steroid hormone biosynthesis, and several adipose tissue development-related pathways were also significantly enriched (Fig. 3C). Involved DEGs include *CYP1A1*, *UGT1A4*, and a large set of known genes with well-characterized pivotal roles in adipocyte proliferation and differentiation (e.g., *ACADL*, *PLIN1*, *RXRG*, *PPARG*, and *FABP5*). Meanwhile, a total of 177 GO terms were markedly overrepresented

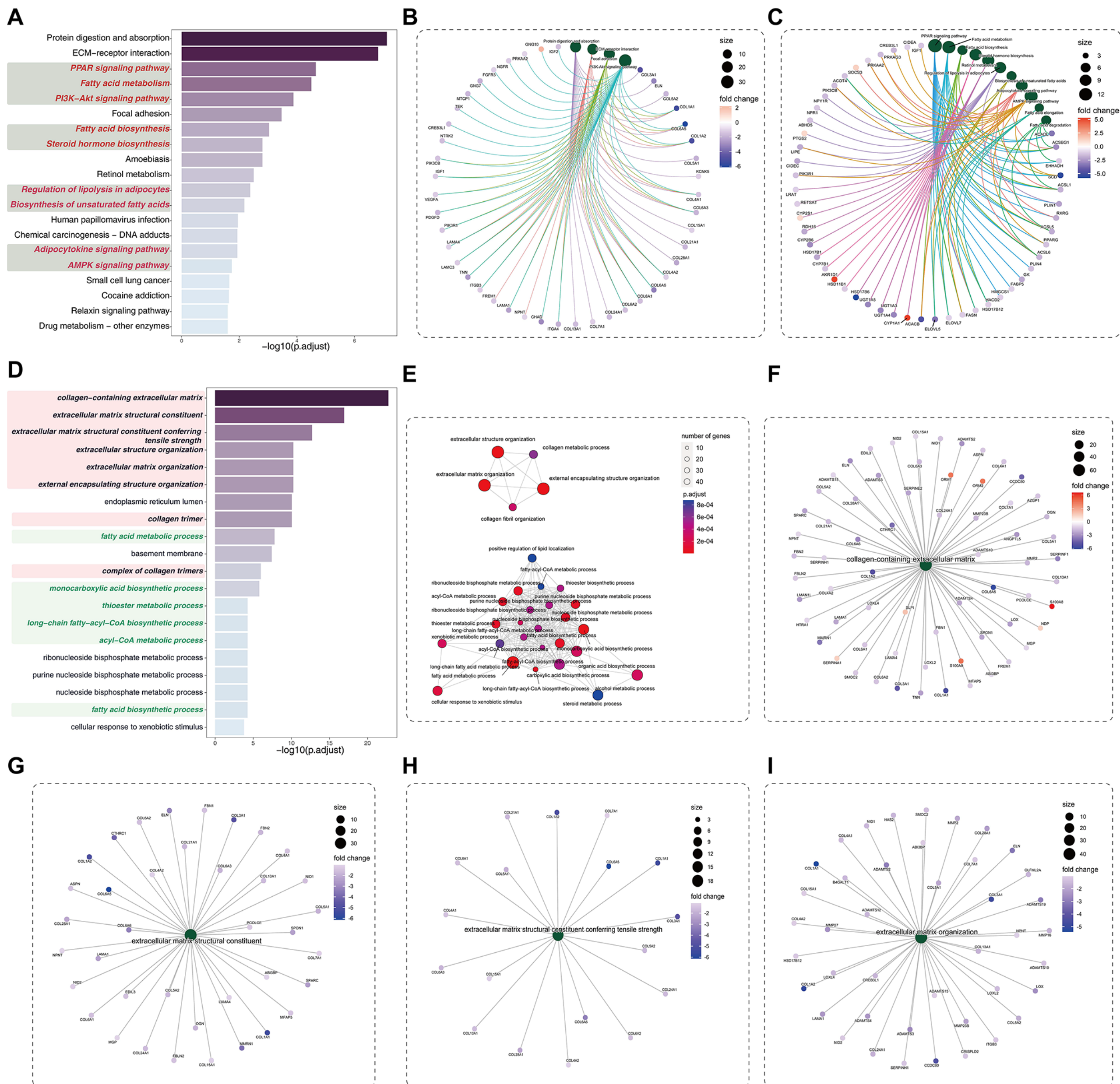

Fig. 3 Functional enrichment of DEGs identified from HG versus HNDG. **A** Top 20 enriched KEGG pathways. **B**-**C** Graphical display of several important pathways and associated genes. **D** Top 20 enriched GO items. **E** Interaction network analysis of GO terms. **F**-**I** Graphical display of pivotal GO terms and associated genes

(Table S4). It is also noteworthy that a group of extracellular matrix and fatty acid biosynthesis-associated items are top ranked (Fig. 3D). Interactive analysis suggested that enriched GO terms markedly form two independent subnetworks (Fig. 3E). One minor network contains extracellular matrix organization, collagen fibril organization, and other terms. The other major subnetwork mainly contains several entries about fatty acid metabolism. We also displayed selected terms and their associated genes. For example, a total of 66 DEGs, the majority of which are overexpressed in HG, participate in the term-collagen-containing extracellular matrix (Fig. 3F). In addition, extracellular matrix structural constituent, extracellular matrix structural constituent conferring tensile strength, and extracellular matrix organization are exhibited (Fig. 3G-J). A dozen genes encoding collagens and extracellular matrix proteins are involved in these terms. For instance, *COL3A1*, *COL5A2*, *COL1A1*, and other collagen-encoding genes take part in extracellular matrix structural constituent conferring tensile strength [33].

Similarly, the KEGG pathway enrichment analysis result indicated that 24 signaling pathways are significantly enriched from DEGs identified from YDMG in comparison with HNDG skin tissues. These pathways include hematopoietic cell lineage, retinol metabolism, graft-versus-host disease, type I diabetes mellitus, and others (Fig. 4A). Three hormone metabolism-related pathways and their associated genes were specifically exhibited (Fig. 4B). Several gene members (e.g., *CYP1A1*) in cytochrome P450 family, *STAR*, and *IGF1* attend above signaling cascades. GO enrichment result suggested that a sum of 136 terms is significantly overrepresented (Table S5). Oxidoreductase activity, monooxygenase activity, collagen-containing extracellular matrix, acute-phase response, and other terms are top-ordered (Fig. 4C). We also found that *ADAMTS2*, *COL1A1*, *COL3A1*, *COL1A2*, *SPARC*, *ELN*, and other genes participate in collagen fibril organization, extracellular matrix organization, extracellular structure organization, collagen-containing extracellular matrix, and other closely related terms (Fig. 4D). Furthermore, several members of cytochrome P450 gene family (e.g., *CYP1A1*), *ACSBG1*, *HACL1*, and other genes are concerned with unsaturated fatty acid metabolic process and other fatty acid metabolism-related GO terms. The above results demonstrated that these enriched signaling pathways and GO terms potentially underly the molecular mechanisms affecting goatskin quality.

Screening of overexpressed genes in meat breeds versus dairy breeds, functional enrichment, and protein-protein interaction network analysis

To further filter functional candidate genes affecting goatskin quality, we intersected the upregulated genes identified from meat breeds vs. dairy breeds. As indicated in Fig. 5A, a total of 106 down-regulated genes were obtained. The expression status of these genes is shown by the heatmap in Fig. 5B and Table S6 (valued by FPKM). Certain genes, including *LOC108634621*, *LOC108634550*, and *LOC108634620*, are specifically expressed in meat goat breeds. Subsequent GO enrichment analysis demonstrated that 35 terms are significantly overrepresented (Table S7). Top listed entries contain extracellular matrix organization, extracellular structure organization, external encapsulating structure

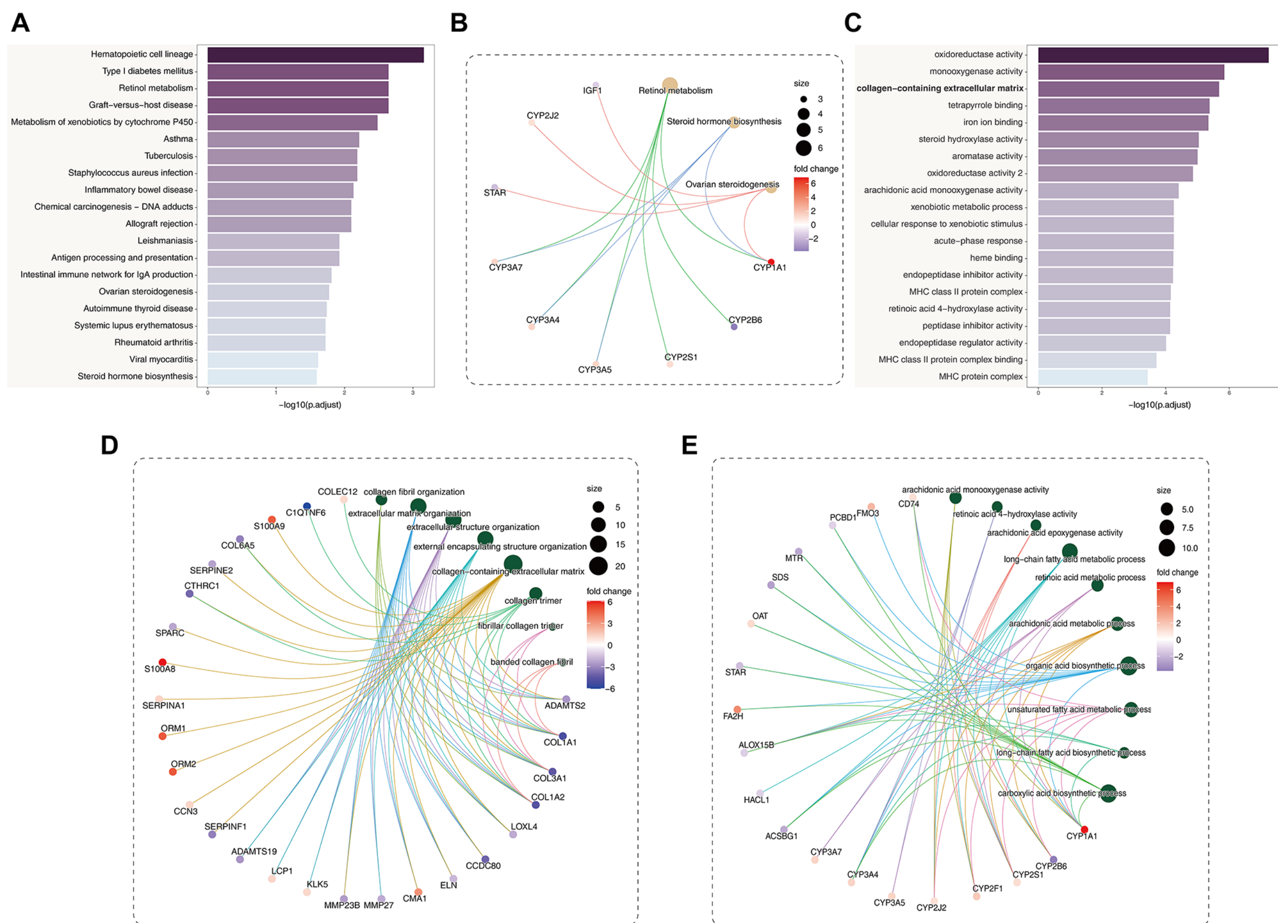

Fig. 4 Functional enrichment of DEGs identified from YDMG versus HNDG. **A** Top 20 enriched KEGG pathways. **B** Graphical display of hormone-related pathways and involved genes. **C** Top 20 enriched GO items. **D**-**E** Graphical display of selected GO terms and associated genes

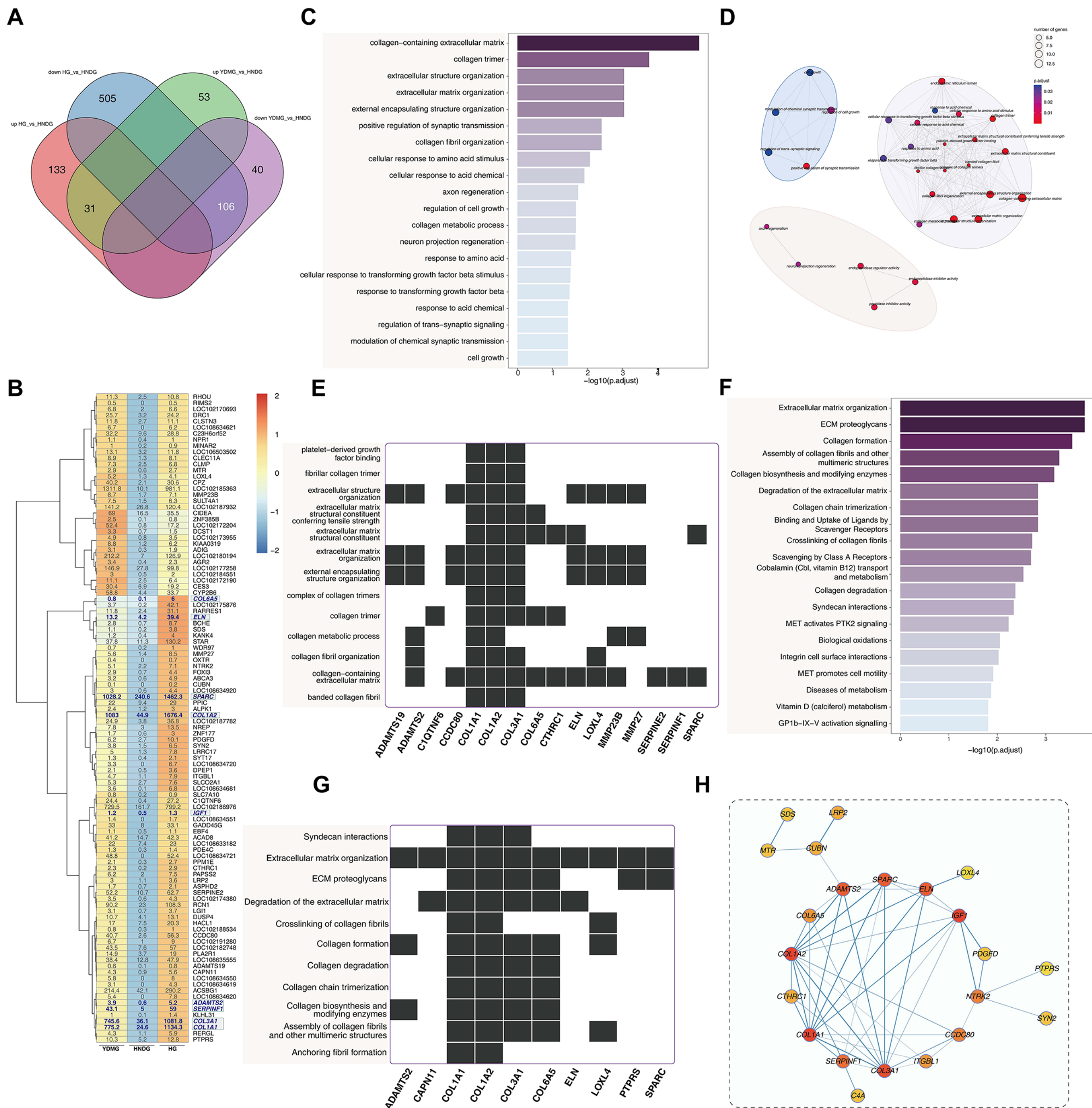

Fig. 5 Screening, functional enrichment analysis, and protein-protein interaction (PPI) analysis of co-upregulated DEGs in meat goats versus dairy goat. **A** A total of 106 co-upregulated genes were identified. **B** Heatmap showing the expression of 106 gene in three goat breeds. **C** Top 20 enriched GO items. **D** Interaction network analysis of GO terms. **E** Graphical display of selected GO items and associated genes. **D** Top 20 enriched Reactome items. **E** Graphical display of selected Reactome items and associated genes. **F** Protein-protein interaction (PPI) analysis of genes

organization, collagen trimer, extracellular matrix structural constituent collagen fibril organization, and others (Fig. 5C). GO terms network analysis revealed three individual subnetworks emerge, among whom collagen and extracellular matrix related terms constitute the major one (Fig. 5D). Selected GO terms and their affiliated genes are shown in Fig. 5E. For example, *COL1A1*, *COL1A2*, *COL6A5*, and *COL3A1* participate in the term-extracellular matrix structural constituent conferring tensile strength [34]. In addition, 31 Reactome terms are significantly enriched (Table S6). Extracellular matrix organization, ECM proteoglycans, collagen formation, assembly of collagen fibrils and other multimeric structures, collagen biosynthesis, and modifying enzymes, and others are top listed (Fig. 5F). Interested terms and associated genes are displayed in Fig. 5G. For instance, *SPARC*

participates in the two terms- Extracellular matrix organization and ECM proteoglycans. Protein-protein interaction (PPI) network analysis result hinted that COL1A1, COL1A2, COL3A1, ELN, SPARC, IGF1, and ADAMTS2 are the core nodes in the network (Fig. 5H), indicating their extensive interactions with other proteins and potential crucial roles in affecting goatskin quality.

qRT-PCR validation of RNA-seq data

To validate the reliability of RNA-seq data, we selected seven genes and checked their relative expressions among three groups using qRT-PCR. Obtained result showed that the relative abundances of all genes show consistent trends with their read counts among three groups (Table S8), indicating the high credibility of sequencing data.

Discussion

Although goatskins are valuable by-products in goat production, the factors and genetic mechanisms impacting their quality are not extensively studied. Herein, we performed ultrastructural and transcriptomic analysis of skin tissues from three goat breeds with distinct production directions. These results hinted potential factors, genes, and molecular mechanisms involved in determining goatskin quality, which is useful for genetic resource conservation and genetic improvement of local goat breeds. Moreover, these observations are helpful for deeper investigation of biomechanical traits of hides in other livestock.

Generally, mammalian skin comprises multiple structural layers, including exterior epidermis, underlying dermis and hypodermis, and several types of appendages, such as hair follicle (HF) [35]. Distinct developmental and growth patterns of HF, which result in diverse hair phenotypes, are caused by drastic global gene expression differences in skin tissues, as previously explored on cashmere and dairy goats [36]. Except for the highly similar coat composition via visual examination (Fig. 1A), a few genes and signaling pathways (e.g., Wnt and BMP) involved in HF development were identified from comparative transcriptomic analysis in our study. Thus, the above findings minimized the possibility that transcriptomic differences in skin tissues between meat and dairy goat breeds are caused by HF development. In addition, the diameter of collagen fibril is a critical factor influencing the biomechanical features of skin, and the hide quality of several livestock species [37]. However, we did not find significant differences regarding the fibril diameters of HG and HNDG in the present experiment. This observation fits with past literature on sheep but is at odds with earlier reports on cattle [11]. Several studies verified that other factors, including collagen fiber organization, collagen crosslink, and the types and concentrations of glycosaminoglycans, are important determinants of skin physical properties [10, 37]. For example, Sizeland et al. reported that collagen fibril orientation is linearly correlated to the tear strength of leather in several mammal species [38]. Therefore, ongoing investigation of parameters related to skin collagen fibril orientation and other factors in various goat breeds should be the focus of the following studies.

It is generally accepted that breed or genotype is a factor of primary importance that affects the physico-mechanical properties and quality of leathers in livestock, including goat [4], sheep [6], cattle [7], and rabbit [8]. Our study demonstrated that breed greatly influences the gene expression patterns in skin tissues, as suggested by more DEGs being screened in meat versus dairy goat breeds than goat breeds themselves. Based on our previous observations, we inferred that certain DEGs are important regulators of skin development and goatskin quality. As expected, functional enrichment analysis results of DEGs powerfully validated such supposition. For instance, several GO terms related to the physical properties of skin, including collagen-containing extracellular matrix, collagen trimer, and collagen fibril organization, are significantly enriched (Figs. 3D-I and 4D). Moreover, we noticed that a myriad of signaling pathways and GO terms about lipid, retinol, steroid metabolism appeared. In mice, Enser et al. observed that the tensile strength of skin under obese conditions is markedly greater than in lean conditions [39]. However, Pierard et al. stated that skins are more extensible in slim than fat people [40]. Hypodermis is the main site of fat deposition in the skin and adipose can release free fatty acids (saturated and unsaturated) to regulate fibroblast growth and collagen deposition [41]. For example, palmitic acid and arachidonic acid impede skin fibroblast proliferation and collagen synthesis, but eicosatetraenoic acid mainly exerts a stimulatory effect [41–43]. Taken together, adipose deposition and fat acid metabolism should be a critical factor controlling goatskin quality via affecting overlying dermis structure and collagen synthesis. In addition, retinol, the frequently used anti-aging agent, ameliorates skin elasticity by boosting the expressions of collagen and elastin and enhancing the formation of elastic fiber network [15]. Steroid hormones (progesterone and estrogen) are also known to modulate cervical mechanical functions (e.g., tissue strength) by elevating the expressions of genes encoding collagen and elastin and reinforcing the ultrastructure of collagen fiber and elastin fiber [44]. Therefore, these hormones possibly perform as key modulators of skin development and leather quality in goats.

We also identified a group of functional genes specifically expressed in the skin tissues of meat goats. Functional enrichment analysis exhibited that these genes mostly participate in extracellular matrix (ECM) and

collagen-related terms, which control skin structure and biomechanical properties. ECM is a 3-dimensional network constituted by a series of macromolecules, such as collagen, elastin, laminins, and others, conferring distinct tensile strength and elasticity of individual tissues [12, 13]. In humans, natural aging is associated with deterioration of tensile strength [45] and degeneration of ECM integrity caused by reduction of collagen and elastin protein expressions [14]. These reports are highly consistent with the elevated levels of these genes in meat goat breeds (Fig. 5B), which produce goatskins with better biophysical properties. In addition, several genes involved in ECM proteoglycan and collagen fibril crosslink emerged. Dysfunction of SPARC, a collagen-binding matricellular protein, leads to half of the tensile strength reduction in mice skins through regulating the binding of collagen to cell surface receptors and ECM assembly [18, 46]. Lysyl oxidase (LOX) and lysyl oxidase-like proteins (LOXLs) are capable of catalyzing covalent cross-linking of the extracellular matrix (ECM) proteins collagens and elastin, contributing to ECM stiffness and mechanical properties [47]. *LOXL4*, the gene with higher levels in meat breeds, is responsible for pathological collagen crosslinking in human lung fibrosis [48]. Based on the functional enrichment of upregulated genes and the above reports regarding their functions, we guessed that the crosslink and organization rather than the diameter of collagen are factors with pivotal roles in shaping the biomechanical properties of goatskins. Moreover, we found that IGF1 possesses extensive protein-protein interaction with the main components of skin tissues, including collagens, elastin, and SPARC. Stimulatory effects of IGF1 on elastin and collagen expression, and proteoglycan deposition in fibroblasts and articular chondrocytes have been studied [49, 50]. Thus, IGF1 should be a crucial growth factor impacting skin tissue development and leather quality in goats. Subsequent studies are needed to define which signaling pathways are activated during the above process.

Conclusion

Ultrastructural analysis of skin tissues from three goat breeds indicated that collagen fibril diameter is not a major factor affecting goatskin quality. Functional enrichment of DEGs identified among goat breeds revealed that terms related to ECM organization, and the metabolism of hormones (retinol and steroid) and fatty acids potentially involve in shaping goatskin quality. PPI inference hinted collagens (COL1A1, COL1A2, COL3A1), ELN, SPARC, IGF1, and others are core players in determining hide quality. Further ultrastructural studies are needed to examine the differences in ECM organization goatskins, and to clarify the characteristics of adipose, hormones, and growth factors in goat skin tissue organization.

Abbreviations

ACADL	acyl-CoA dehydrogenase long chain
ACSBG1	acyl-CoA synthetase bubblegum family member 1
ADAMTS2	ADAM metallopeptidase with thrombospondin type 1 motif 2
BCHE	butyrylcholinesterase
BMP	bone morphogenetic protein
CCDC80	coiled-coil domain containing 80
COL1A1	collagen type I alpha 1 chain
COL1A2	collagen type I alpha 2 chain
COL3A1	collagen type III alpha 1 chain
COL5A2	collagen Type V Alpha 2 Chain
CHI3L2	chitinase 3 like 2
CYP1A1	cytochrome P450 family 1 subfamily A member 1
CUBN	cubilin
DEGs	differentially expressed genes
ECM	extracellular matrix
ELN	elastin
FABP5	fatty acid binding protein 5
FPKM	fragments per kilobase of exon model per million mapped fragments
GABRA3	gamma-aminobutyric acid type A receptor subunit alpha3
GO	gene ontology
HACL1	2-hydroxyacyl-CoA lyase 1
IGF1	insulin-like growth factor 1
KEGG	Kyoto encyclopedia of genes and genomes
LOX	lysyl oxidase
LOXLs	lysyl oxidase-like proteins
NRP1	natriuretic peptide receptor 1
PDGFD	platelet-derived growth factor D
PLIN1	Perilipin 1
PPARG	peroxisome proliferator-activated receptor gamma
RXRG	retinoid X receptor gamma
SEM	scanning electronic microscopy
SERPINF1	serpin family F member 1
STAR	steroidogenic acute regulatory protein
SYN2	synapsin II
SPARC	secreted protein acidic and cysteine rich
UGT1A4	UDP glucuronosyltransferase family 1 member A4
VEGFA	vascular endothelial growth factor A

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10995-8.

Supplementary Material 1: Table S1 Selected genes and primers

Supplementary Material 2: Table S2 Summary of sequencing data

Supplementary Material 3: Table S3 Global expression levels of genes

Supplementary Material 4: Table S4 Differentially expressed genes between groups

Supplementary Material 5: Table S5 KEGG and GO enrichment analysis of DEGs between HG and HNDG

Supplementary Material 6: Table S6 KEGG and GO enrichment analysis of DEGs between YDMG and HNDG

Supplementary Material 7: Table S7 Co-downregulated genes and functional enrichment analysis

Supplementary Material 8: Table S8 qRT-PCR validation of gene expressions

Acknowledgements

The authors would like to thank the owners and employees for their kind help in the process of sample collection.

Author contributions

SM and XWW conceived the design of the study. XLH, SH, and JYD conducted the experiments, and SM and JXT performed the bioinformatic analyses and

interpreted the data. SM, YSH, ZJX, ZML, and XWW prepared and revised the manuscript. All authors read and approved the final manuscript.

Funding
This work was supported by the Modern Agricultural Production System of Henan Meat Goat and Sheep (HARS-22-15-Z1), the National Natural Science Foundation of China (31872332), and the Science and Innovation Training Program for College Students in the College of Animal Science and Technology of HAU (DK202201).

Data availability
Sequence data that support the findings of this study have been deposited in the China National Center for Bioinformation database as BioProject No. PRJCA019523 and GSA NO. CRA012501.

Declarations

Ethics approval and consent to participate
All experimental methods were performed by the relevant guidelines, regulations, and legislation.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 7 June 2024 / Accepted: 5 November 2024
Published online: 11 November 2024

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