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Single-cell transcriptomic profiling of goat milk somatic cells highlights immune heterogeneity and epithelial cell-related networks

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ABSTRACT

The global goat milk market has expanded rapidly, driven by its reputed hypoallergenic properties and associated health benefits. Here we present the first single-cell RNA sequencing (scRNA-seq) atlas of somatic cells in mid-lactation Saanen goat milk, revealing cellular heterogeneity and immune-regulatory mechanisms. Analysis of 7276 high-quality cells from five biological replicates revealed seven populations: myofibroblasts, dendritic cells (DCs), epithelial cells (EPCs), monocytes, bone marrow-derived progenitor cells, neutrophils, and T cells. The proportion of EPCs varied markedly between individuals (23.18 %–94.09 %, $p = 0.0295$) and was positively correlated with somatic cell count (R = 0.6087, based on five biological replicates), suggesting a moderate association. Pseudotime analysis revealed two differentiation trajectories: high-epithelial samples (HPG; >80 % EPCs) were dominated by immune-cell- dominated, whereas low-epithelial samples (LPG; <60 % EPCs) primarily exhibited epithelial differentiation. Cell-cell communication analyses showed distinct signaling: SELL/CXCL-mediated immune pathways were activated in HPG, while TGF-β/SPP1 signaling—linked to cell migration and immune suppression—was upregulated in LPG. A total of 214 differentially expressed genes (DEGs) were identified. Pro-inflammatory factors, such as *SAA* and *PAEP*, were enriched in HPG, whereas anti-inflammatory markers, including *SERPIN B3* and *C3*, were elevated in LPG. Notably, casein genes (*CSN1S2, CSN2*, *CSN3*) were markedly upregulated in immune cells of HPG (T cells, monocytes and DCs). In conclusion, this work unveils a key cellular biomarker for milk quality, which is expected to guide the dairy industry towards producing safer and hypoallergenic goat milk products.

1. Introduction

The global goat milk market has expanded rapidly, primarily owing to its the nutritional advantages of goat milk compared with cow's milk (Clark & García, 2017; Miller & Lu, 2019). Goat milk is often reported to be less allergenic and is rich in oligosaccharides—such as 6′-sialyllactose, 2′-fucosyllactose, and 3-fucosyllactose—which can selectively promote the growth of beneficial gut microbiota. Such prebiotic activity has been associated with improved immune function, beneficial effects on infant neurodevelopment, and reduced intestinal inflammation (Van Leeuwen et al., 2020; Wang et al., 2018). Smaller fat globules and more easily digestible proteins in goat milk may facilitate nutrient absorption compared with cow's milk, including improved uptake of minerals such as calcium, phosphorus, and iron (Campos et al., 2003; Felice et al., 2021; Liu HongYan, Guo XiaoQing, Zhao QingYu, Qin YuChang, & Zhang JunMin, 2020). Recent studies have identified nearly 40 bioactive peptides in goat milk—including antimicrobial, ACE-inhibitory, and immunomodulatory compounds—compared to only 10 detected in cow's milk (Hodgkinson et al., 2019).Furthermore, goat milk contains immunomodulatory components such as DEFB1, S100A7, and cathelicidin-2, which exhibit antibacterial, immunomodulatory, cholesterol-lowering, antioxidant, and DPP-IV inhibitory

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activities (Mohanty, Mohapatra, Misra, & Sahu, 2016 Sep; Zhang, Chen, et al., 2015). Collectively, these findings suggest an important role for goat milk in gut health, immune modulation, and metabolic regulation.

Numerous key genes associated with goat milk quality and yield have identified. For instance, *MST1* and *ADIPOR1* have been implicated in regulating lipid metabolism in goat mammary epithelial cells (EPCs) through different pathways (Chen et al., 2018; Zhao et al., 2021). In relation to lactation, *PRLR*, *LALBA*, and *IGF-IR* significantly affect milk yield and protein composition (Hou et al., 2013; Luo et al., 2019). The casein gene family (*CSN1S1*, *CSN1S2*, *CSN2*, *CSN3*) encodes the major milk proteins and influences yield, composition, cheese processing properties, as well as the digestibility and allergenicity of dairy products (Kruchinin et al., 2023; Ma et al., 2020). Moreover, the number and composition of milk somatic cells—largely leukocytes—are sensitive indicators of mammary gland health and play key roles in defending against pathogenic invasion (Alhussien & Dang, 2018; Pegolo, Giannuzzi, et al., 2021; Rainard et al., 2018). Increases somatic cell count (SCC) is associated with reduced milk fat content, decreased cheese yield, flavor deterioration, and shortened shelf life, among other quality issues (Park & Humphrey, 1986; Pegolo et al., 2021). However, the heterogeneity of milk somatic cells and their underlying regulatory mechanisms genetic regulatory mechanisms remains poorly understood.

RNA sequencing (RNA-seq) has been widely used to study gene expression in mammary tissue and milk somatic cells of goats (Sahito et al., 2023; Song et al., 2024; Crisà et al., 2016). However, conventional methods that analyze bulk tissues cannot resolve cell type-specific expression patterns. The development of single-cell RNA sequencing (scRNA-seq) addresses this limitation, allowing for the analysis of transcriptomic heterogeneity (Cheng, Chen, Jin, & Chen, n.d.; Jovic et al., 2022), the study of tissue development mechanisms(Cai et al., 2023; He et al., 2023), and the identification of disease pathways (Yamada & Nomura, 2020).

ScRNA-seq has also advanced food science by enabling assessment of how food-derived compounds influence cellular responses and potentially contribute to health outcomes (Wang et al., 2024). For example, scRNA-seq profiling revealed that dietary antigens can modulate tumorigenesis via immune effects in the human small intestine (Sasaki et al., 2024). In animal food-science studies, scRNA-seq has characterized lineage diversity and markers for intramuscular fat in chicken breast (Li et al., 2020); identified adipocyte subpopulations contributing to intramuscular fat in pigs (Xu et al., 2025); and defined developmental trajectories of scallop adductor muscle cells (Sun et al., 2021). In dairy research, scRNA-seq has proven particularly valuable by resolving functional subsets in human and bovine milk cells, elucidating lactation-related gene regulatory networks, and mapping the molecular landscape of mammary cells populations during lactation (Saager et al., 2024; Zorc et al., 2024). However, functional heterogeneity and immune signaling networks of milk somatic cells at single-cell resolution remain largely unexplored in goats.

This study employed scRNA-seq to systematically characterize the somatic cell types and transcriptional programs within goat milk somatic cells during mid-lactation. This approach facilitates a deeper understanding of the molecular features and functions of diverse cell populations, providing new insights that may improve dairy product safety and support the development of functional dairy products.

2. Materials and methods

2.1. Ethical statement and sample collection

This study was approved by the Animal Experiment Ethics Committee of Southwest University (Ethical Review No.IACUC-20240710-04). Five healthy 14-month-old Saanen dairy goats in mid-lactation (From 100 to 115 days in milk) were selected from the Southwest University goat breeding base (Longitude 105.295153, Latitude: 29.343887, Altitude: 248 m). Animals were maintained on a 6:4 concentrate-to-forage diet. Milk yield was recorded twice daily at 07:00 and 18:00. After six consecutive days of milking, 100 mL of milk was collected from each goat on day 7 for somatic cell isolation. Prior to sampling, teats were disinfected and milk was collected into sterile containers.

2.2. Somatic cell isolation, single-cell library construction, and quality control

At 20 °C, Goat milk samples were diluted 1:1 with sterile phosphate-buffered saline (PBS) and centrifuged at 900×*g* for 20 min. Following the removal of the supernatant, the pellet was resuspended in 10 mL of PBS and subjected to a second centrifugation under the same conditions for 8 min to enrich somatic cells (Golan et al., 2025). SCC was measured using a LUNA-FL™ Dual Fluorescence Cell Counter (Logos Biosystems, South Korea). And the viability of all sample cells is higher than 85 %. The fragmentation rate is less than 4 %. The agglomeration rate is less than 5 %. To potential contamination from residual epithelial cells or transcript leakage during analysis during subsequent analysis on data accuracy. The milk somatic cells suspension was loaded onto the 10K Genomics Perseus™ platform (Shanghai, China) for transcriptome library preparation, and sequencing was performed on a NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Raw sequencing data underwent quality control: low-quality reads with a Phred score <10 were removed, and the 5′ transcription start site (TSO) adapter and 3′ poly (A) tail were trimmed using paired-end clipping, retaining only high-quality reads (HQRs). HQRs were aligned to the goat reference genome (ARS1.2 version) using STAR software (v2.5.3; GitHub - alexdobin/STAR: RNA-seq aligner) with default parameters, and PCR duplicates were corrected using unique molecular identifiers (UMIs).

2.3. Analysis of single-cell transcriptome data

Single-cell data were processed using Seurat v4.0 (Butler et al., 2018). Cells with fewer than 200 detected genes or >5 % mitochondrial gene content were excluded. Additionally, genes detected in at least three cells were included for subsequent analysis. Data normalization was performed using the NormalizeData function in Seurat. Principal component analysis (PCA) was then conducted, with the top 30 principal components selected for further analysis. Cell clustering was performed using the Louvain algorithm based on graph theory. To annotate cell clusters, differentially expressed genes (DEGs) for each cluster were first identified using the FindAllMarkers function. The identified marker genes for the different cell clusters were then cross-referenced with the CellMarker database (http://bio-bigdata.hrbmu.edu.cn/CellMarker/).

2.4. Pseudotime analysis

Pseudotime analysis was performed using Monocle (v2.8.0; http://cole-trapnell-lab.github.io/monocle-release/docs/#getting-started-with-monocle). All cell clusters were extracted and further subdivided into distinct cell groups according to marker gene expression. Dimensionality reduction was performed using the DDRTree function, and cell differentiation states were determined using the orderCells function.

2.5. Cell-cell communication analysis and cell signaling pathway mapping

Cell-cell communication analysis and cell signaling pathway mapping were conducted using CellChat (v2.1.2) (Jin et al., 2025). CellChat contains a comprehensive database of ligands, receptors, cofactors, and their interactions (http://www.cellchat.org/). The significance of cell-cell communication was assessed using permutation tests to evaluate each ligand–receptor pair.

2.6. Cell cycle analysis

Cell cycle analysis was performed using the CellCycleScoring function in Seurat (Butler et al., 2018). Based on the expression of established marker genes for each cell cycle phase (S, G1, and G2/M), each cell was assigned a cell cycle score and annotated as G2/M, G1, or S phase.

2.7. Sample grouping

Samples LC56 and HC40, which exhibited an epithelial cell (EPC) content greater than 80 % relative to all cells identified in the single-cell RNA sequencing (scRNA-seq) analysis, were classified as the high-content group (HPG). In contrast, samples HC47, HCS64, and LCS048, which exhibited an EPC content of less than 60 %, were classified as the low-content group (LPG).

2.8. Functional enrichment analysis of DEG and KEGG between groups

Inter-group differential expression gene analysis was conducted through the Seurat FindMarkers function of R (v4.3.1), applying criteria of "|avg_log2FC| > 0.25"and "*p*_val_adj <0.05"to define the significant DEGs. Functional enrichment analysis was conducted using KOBAS (http://bioinfo.org/kobas) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Enrichment significance was determined by the hypergeometric test, and pathways with a *p*-value <0.05 were considered significant.

2.9. Statistical analysis

Quantitative data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using R software (version 4.3.1). Differences in epithelial cell proportions between the HPG and LPG groups were assessed using Welch's two-sample *t*-test. The Pearson correlation coefficient between the epithelial cell proportion (EPC) and SCC for each sample was calculated using the default parameters of the cor() function in R. According to the classification proposed by Schober et al. (2018), correlation strength was interpreted as negligible (0.00–0.09), weak (0.10–0.39), moderate (0.40–0.69), strong (0.70–0.89), or very strong (0.90–1.00). A *p*-value <0.05 was considered

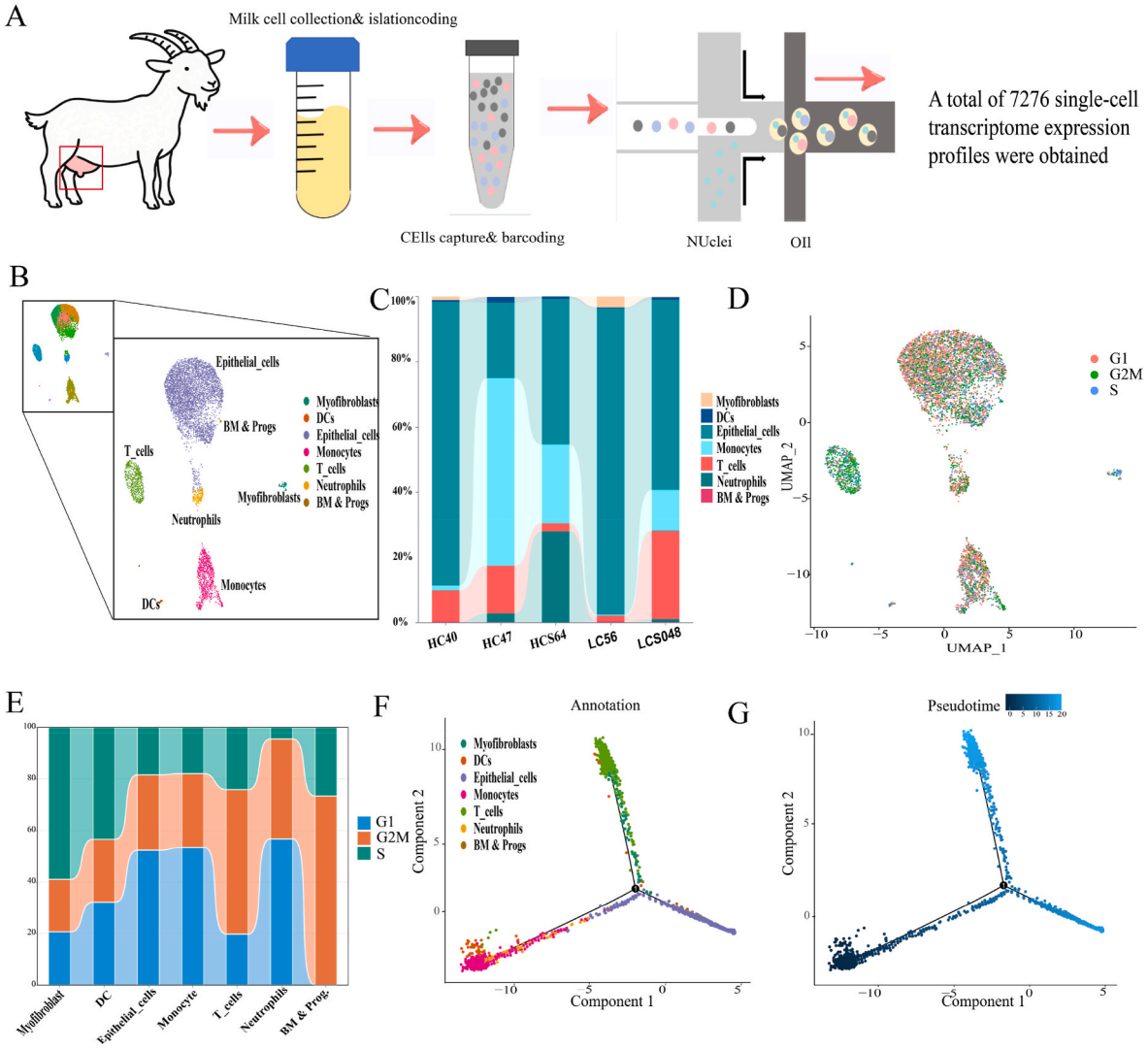

Fig. 1. ScRNA-seq Analysis Results of Five Samples. (A) Overview of the single-cell transcriptomic sequencing workflow for Saanen dairy goat milk samples. **(B)** Cell type annotation, with different colors representing distinct clusters/types. **(C)** Proportional composition of cell types across samples (x-axis: sample names; y-axis: relative abundance of each cell type). **(D, E)** Cell cycle phase distributions: **(D)** Overall profiles across samples (colors denote phases); **(E)** Phase proportions per cell type (x-axis: cell types; y-axis: phase percentages). **(F, G)** Pseudotime analysis: **(F)** Spatial distribution of cell types along the differentiation trajectory; **(G)** Starting point of pseudotemporal ordering.

statistically significant for all tests.

3. Result

ScRNA-seq was performed on five goat milk samples, generating 39.2 Gb of high-quality data and retaining 7276 cells after quality control (Fig. 1A). T-SNE dimensionality reduction and unsupervised clustering identified nine initial cell clusters (Fig. 1B). These clusters were annotated as seven cell types based on established marker genes (Table S1): myofibroblasts, dendritic cells (DCs), EPCs, monocytes, bone marrow-derived progenitor cells (BM&Progs), neutrophils, and T cells (Fig. 1B). EPCs formed the largest fraction (61.47 % ± 21.48 %),

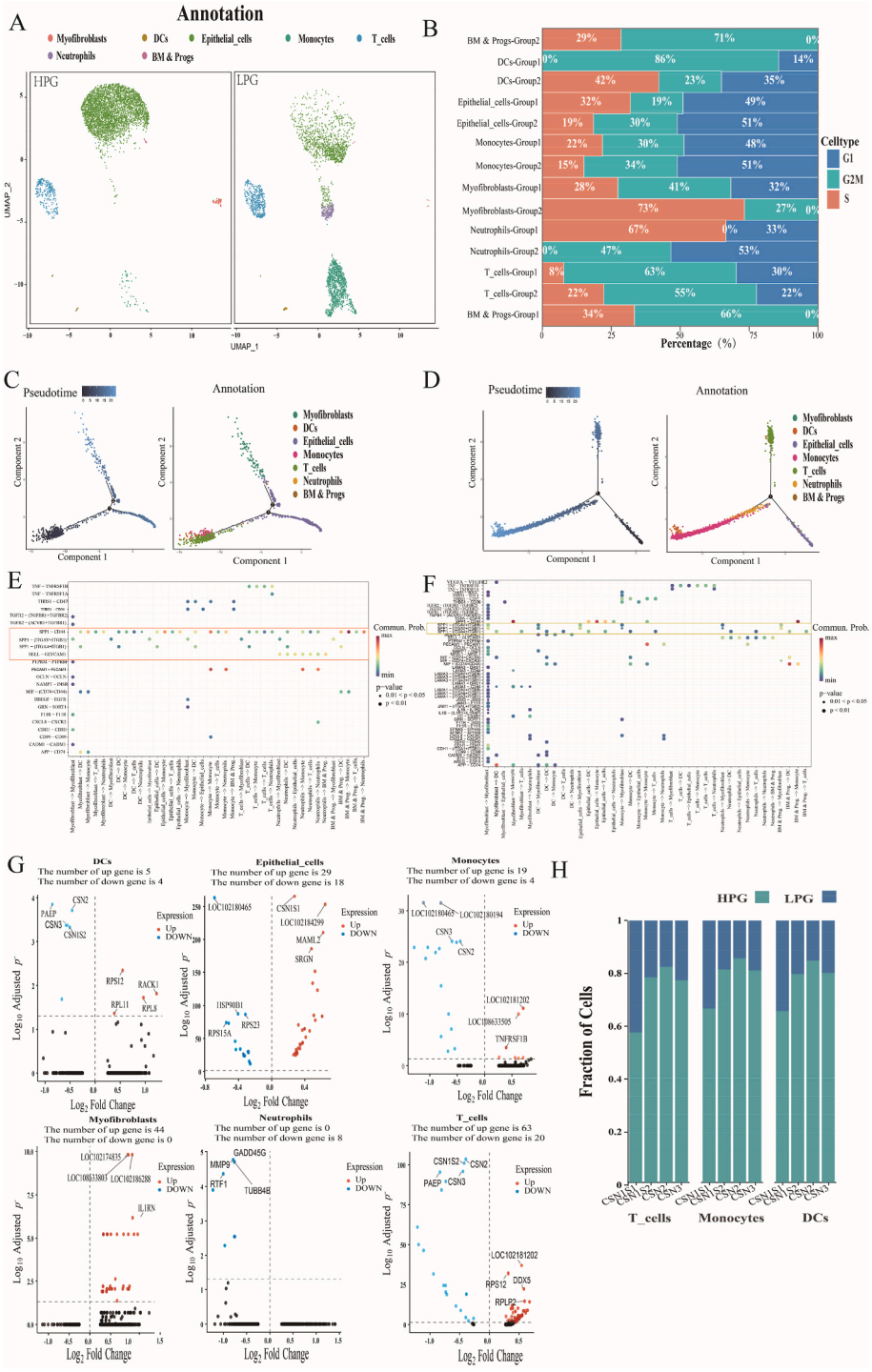

Fig. 2. An analysis of cell-cell communication, cell differentiation, and differential gene expression between the two groups after grouping. **(A)** Cell type composition and proportions in HPG and LPG (HPG: high epithelial cell content; LPG: low epithelial cell content). **(B)** Cell cycle analysis by group and cell type (x-axis: phase proportions; y-axis: groups and cell types; colors indicate phases). **(C, D)** Pseudotemporal differentiation trajectory for HPG (C) and LPG (D) groups. **(E, F)** Cell-cell communication diagrams for HPG (E) and LPG (F) groups (x-axis: cell types and communication directions; y-axis: cell pathways; dot color: communication intensity; dot size: significance; SPP1-related pathways highlighted). **(G)** Distribution of DEGs by cell type (Upregulated: higher in LPG; Downregulated: higher in HPG; top four upregulated/downregulated DEGs in each cell type highlighted). **(H)** Relative expression levels of casein family genes in immune cells (T cells, monocytes, DCs) in HPG and LPG.

whereas BM&Progs were the least abundant (0.16 % ± 0.14 %) (Fig. 1C–Table S2). EPCs proportion varied substantially among samples (23.18 %–94.09 %) and was positively correlated with the SCC (R = 0.6087, Table S3).

Cell cycle analysis showed that all cell types, except BM&Progs (which were restricted to G1 and S phases), spanned the G1, S, and G2/M phases. Neutrophils, BM&Progs, and myofibroblasts showed the highest proportions in the G2/M, G1, and S phases, respectively (Fig. 1D and E). Pseudotime analysis placed monocytes near the trajectory root, which bifurcated into two branches: one primarily composed of T cells and myofibroblasts, and the other dominated by epithelial cells (EPCs) (Fig. 1F and G). These results indicate that monocytes, EPCs, T cells, and myofibroblasts represent discrete cellular states along the inferred pseudotemporal path.

Grouping samples by EPC proportion revealed a significant difference between HPG and LPG ($p = 0.0295$) (Fig. 2A). Significant differences in cell cycle distribution were observed for BM&Progs, myofibroblasts, and neutrophils between groups ($p < 0.05$). For instance, the S-phase proportion of myofibroblasts was 58.02 % in the HPG but reached 100 % in the LPG (Fig. 2B). Differentiation trajectories differed between groups: in HPG, monocytes and T cells occupied early pseudotime positions, followed by myofibroblasts and EPCs (Fig. 2C). In contrast, the pseudotime trajectory in LPG began with EPCs, which were followed by T cells and monocytes (Fig. 2D). Cell-cell communication analysis identified that only a few pathways, such as secreted phosphoprotein 1 (SPP1) and selectin L (SELL), were broadly expressed in both groups. In HPG, immune cells (monocytes and neutrophils) showed frequent interactions mediated by TNF and CXCL pathways. In LPG, signaling via TGF-β1/ACVR1 and LAMA1/ITGA2 in myofibroblasts was significantly increased (Fig. 2E and F).

DEG analysis revealed the distribution of DEGs and their associated functional pathways across cell types in both EPCs groups. Among the seven cell types, only BM&Progs exhibited no detectable DEGs. A total of 214 DEGs were identified across the other six cell types (Fig. 2G). T cells displayed the largest number of DEGs (83), whereas neutrophils had the fewest (8) (Fig. 2G–Table S4). In LPG, 44 upregulated DEGs in myofibroblasts were enriched in 41 KEGG pathways, including ABC transporters, endocytosis, and cytokine-cytokine receptor interaction. In EPCs, 29 upregulated DEGs were enriched in 68 KEGG pathways, with significant enrichment in immune-related pathways, such as phagosome, antigen processing and presentation, and Staphylococcus aureus infection. In monocytes, four upregulated DEGs were enriched in two KEGG pathways: lysosome and ribosome. In DCs, five upregulated DEGs were enriched in two pathways: ribosome and measles (Table S5).

By contrast, in HPG, 18 upregulated DEGs in EPCs were enriched in 16 pathways, including protein processing in the endoplasmic reticulum, ribosome function, and protein export. In neutrophils, eight upregulated DEGs were associated with 40 pathways, including p53 signaling, leukocyte trans-endothelial migration, and estrogen signaling. Nine upregulated T cells DEGs were enriched in 13 pathways, such as inflammatory bowel disease, B cells receptor signaling, Th17 cell differentiation, and Epstein-Barr virus infection ($p < 0.05$, Fig. 2G–Table S6).

These findings reveal how different cell populations respond to external stimuli via distinct molecular mechanisms, including cell-cell communication regulation, immune response, protein metabolism, and signal transduction, under both LPG and HPG conditions. Notably, several DEGs in immune cells (DCs, T cells, and monocytes) from HPG were significantly enriched in the prolactin signaling pathway. The expression levels of casein family genes (*CSN1S2*, *CSN2*, *CSN3*) were also significantly higher in the HPG compared to the LPG (Fig. 2G and H, Table S7).

4. Discussion

This study is the first to elucidate the mechanism underlying the association between compositional differences in milk-derived immune cells and allergenicity. Previous research demonstrates that macrophages enriched in cow's milk secrete pro-inflammatory factors, such as IL-6 and TNF-α (Sladek & Rysanek, 2010). Additionally, studies show that B cells promote IgE production (Boonpiyathad et al., 2019; Choi et al., 2020), and those natural killer cells facilitate the Th2-type immune response (Martorell-Aragonés et al., 2015). Collectively, these immune cells constitute a multi-layered allergenic network. Notably, this study found that the absence of these three immune cell types in goat milk may reduce allergen presentation and the IgE cascade reaction, offering a possible immunological explanation for lower milk allergenicity.

The proportion of EPCs in goat milk (23.18 %–94.09 %) exhibited biological characteristics similar to those observed in human milk (50 %–98 %) (Trend et al., 2015; Yamada & Nomura, 2020), but differed significantly from those found in cow's milk (2.1 %–70 %) (Becker et al., 2021; Hassiotou & Geddes, 2015). Correlation analysis showed a positive association between the proportion of EPCs and SCC, consistent with the pathological process in which increased cell shedding follows epithelial barrier disruption during mammary gland infection (Boutinaud & Jammes, 2002; Brooker, 1980). SCC is a key indicator of mammary gland health (Fonseca et al., 2025), with elevated levels typically reflecting infection (such as subclinical mastitis) or inflammatory states in mammary tissue (Mukasafari et al., 2025; Zhang et al., 2020). The EPCs detected in milk primarily originate from mammary-gland EPCs. Their abundance reflects the integrity and infection status of the mammary epithelium, the gland's physiological state, and management practices. This suggests that EPCs proportion may indicate mammary-gland health status and the immune activity of cells. Milk with a lower proportion of epithelial cells may potentially reflect better mammary-gland health compared to milk with a higher proportion. However, this phenomenon requires further confirmation with larger sample sizes.

This study further explored the cellular differentiation mechanisms contributing to EPCs proportion differences. Single-cell pseudotime analysis revealed that the high EPCs group (HPG) follows an "immune-mesenchymal" differentiation pathway, in which monocytes and T cells are first activated, followed by myofibroblasts and EPCs. In contrast, the low EPCs group (LPG) follows an "epithelium-dominant" pathway. Cell-cell communication analysis showed that heterogeneity in differentiation is driven by distinct activation patterns of key signaling pathways. In LPG, myofibroblasts induce cell cycle arrest by inhibiting the G1/S phase transition via the TGF-β signaling pathway (Jahangirimoez et al., 2020; Petrov et al., 2008), which regulates the epithelial–mesenchymal transition (EMT) process through Smad2/3 phosphorylation (Wang et al., 2023; Xu et al., 2009). In HPG, monocytes and T cells generate a pro-inflammatory microenvironment through L-selectin (SELL)–mediated signaling, secretion of inflammatory factors (IL-6/TNF-α) associated with CD99 (Takheaw et al., 2019; Zhang et al., 2015)), and cell proliferation driven by EGF (Yao et al., 2012). Notably, significant activation of the SPP1 signaling axis in the LPG, especially via SPP1-ITGAV/ITGB1 and SPP1-ITGA4/ITGB1 receptor complexes, suggests an immune regulatory mechanism different from the HPG. This pathway may promote EPCs adhesion and polarization of immunosuppressive macrophages (Stenhouse et al., 2019; Wang, Chen, et al., 2024; Zhang et al., 2016), supporting epithelial homeostasis and immune tolerance in the LPG, in contrast to the pro-inflammatory state in the HPG.

This study also revealed contrasting gene-expression signatures indicative of pro-inflammatory (HPG) versus anti-inflammatory (LPG) regulatory states. In the HPG, pro-inflammatory genes expression in immune cells (such as DCs, monocytes, and T cells) was significantly upregulated, including *LOC102168428* (*SAA*), *PAEP*, and *ELF5*.

Upregulation of *SAA* is known to exacerbate inflammation via the IL-1β pathway (Chang et al., 2025; Lopez-Castejon et al, 2011) *PAEP* suppresses immune defense in non-small cell lung cancer by targeting

glycoproteins (Wang & Kong, 2024). *ELF5* expression has been associated with increased myeloid infiltration and inflammation in luminal A breast cancer (Gallego-Ortega et al., 2015). Notably, EPCs in the HPG exhibited high expression of *HSPA5* and *HSP90B1*; *HSPA5* maintains cell function under hypoxic stress (Wang et al., 2017), whereas *HSP90B1* exacerbates inflammatory damage by upregulating IL-6 and IL-8 (Huang et al., 2025). In contrast, the LPG exhibited anti-inflammatory characteristics. Several anti-inflammatory genes were upregulated in LPG EPCs, including *LOC102184299* (*SERPIN B3*), *C3*, and *KRT19*. *SERPIN B3* reduces inflammatory infiltration by inhibiting protease activity (Lunardi et al., 2011); *C3* protects tissues by enhancing barrier function (Sahu et al., 2023); and *KRT19* restricts immune cell activity by regulating immunosuppressive molecules (Sun et al., 2025).

Previous studies have shown that mammary gland epithelial cells dynamically regulate the immune response by secreting pro-inflammatory factors, such as TNF-α, IL-6, and IL-8, as well as the anti-inflammatory cytokine IL-10 (Furman et al., 2019; Rojas et al., 2017). Cytokines, including IFN-γ and TNF-α from T cells, can activate the macrophage–epithelial cell antibacterial axis (Meisgen et al., 2014). This study observed upregulation of multiple genes associated with T cells immune activation (e.g., *PAG1*, *TANK*, *DUSP22*) in the HPG. *PAG1* negatively regulates SRC family kinases by recruiting Csk, which enhances T cells receptor (TCR) signaling, promotes T cells activation, and amplifies pro-inflammatory effects. High *PAG1* expression is also associated with increased allergy risk (Brian IV et al., 2022; Vicente et al., 2015). *DUSP22* inhibits Lck activation and pro-inflammatory factor expression by dephosphorylating *UBR2* (Shih et al., 2024). *TANK* enhances inflammatory cytokine production by activating the NF-κB pathway, thereby boosting antipathogen immunity (Runde et al., 2022; Zecchini et al., 2023). *RORA* relieves T cells immune suppression by inhibiting programmed death-ligand 1 (PD-L1) and promoting local immune responses (Kalim et al., 2024; Liu et al., 2024). ZFP36L2 regulates cytokines such as IFN-γ and influences the functional persistence of $CD8^+$ T cells (Son et al., 2019; Zandhuis et al., 2024). Although CD21 mainly affects B cells, its interaction with T-bet may mediate T–B cells cooperation, amplifying the immune response (Unger et al., 2018).

In summary, these gene-expression changes can disrupt immune homeostasis, drive production of inflammatory mediators, and lead to cytotoxic damage and pathological immune responses (Bradford & Swartz, 2020). Such disturbances directly compromise the mammary epithelial barrier, leading to inflammatory damage to mammary tissue and a reduction in milk quality.

The immune function is closely associated with dietary nutrients, such as whey protein, and bioactive substances, including bioactive peptides and cytokines (Basak & Gokhale, 2022). Research has shown that both human and donkey milk exhibit substantial anti-inflammatory effects, primarily through the regulation of leukocyte activity, modulation of cytokine signaling, and the release of specific bioactive components. For example, lysozyme in donkey milk improves intestinal function by inhibiting inflammatory responses and stimulating lymphomonocytes to secrete anti-inflammatory cytokines (Buescher, 2001; Li et al., 2022). Human milk, rich in antibacterial factors such as lysozyme and antiproteases, also contains anti-inflammatory cytokines (e.g., IL-4, IL-10, and TGF-β2) that suppress inflammatory responses and help maintain immune homeostasis (Goldman, 2019; Wynn, 2003). Notably, the concentrations of key anti-inflammatory factors (e.g., IL-10 and IL-13) in the milk of mastitis patients are significantly lower than those in healthy individuals (Castro et al., 2022), suggesting that dynamic changes in milk-derived immunomodulatory substances could reflect the health status of the mammary gland.

In this study, we found that differences in EPCs proportions in goat milk may be regulated by the TGF-β/SMAD signaling pathway, which in turn influences the expression profile of immune-related genes. In LPG, anti-inflammatory genes are predominantly expressed, whereas the HPG showed enrichment of pro-inflammatory genes. These differentiation programs may affect mammary tissue repair by regulating myofibroblast proliferation and modulating immune balance (anti-inflammatory versus pro-inflammatory). Therefore, we propose that the proportion of EPCs in goat milk could serve as a novel biomarker for assessing mammary gland health and the immunomodulatory potential of dairy products.

Notably, in the HPG, expression of casein family genes (*CSN1S1*, *CSN1S2*, *CSN2*, *CSN3*) were significantly upregulated in immune cells such as monocytes and DCs. This suggests that casein genes may affect the milk microenvironment through their combined effects on immune regulation and the biological properties of dairy products. Among these, *CSN1S1* (αS1-casein) is recognized as a principal allergen in milk protein allergy (Ballabio et al., 2011; Schulmeister et al., 2008). Its high expression in this study appears to may contribute to the pro-inflammatory characteristics of the HPG, including activation of the IL-1β pathway (Lopez-Castejon et al, 2011). Previous studies have shown that *CSN1S1* contains antigenic epitopes recognized by human IgE and can promotes monocyte differentiation and secretion of pro-inflammatory factors such as GM-CSF and IL-1β (Song et al., 2020; Vordenbäumen et al., 2013). These effects are consistent with the over-activated immune-cell phenotype observed in HPG. Additionally, *CSN2* and *CSN3*, which regulate milk composition and processing properties (Bonfatti et al., 2010; Dettori et al., 2023), are highly expressed in models of Staphylococcus aureus infection and autoimmune diseases (Otaegui et al., 2007; Yagdiran et al., 2016). This evidence supports the notion that casein family genes may intensify inflammatory responses by modulating immune function.

Based on these findings, we propose that casein expressed by immune cells in HPG milk may act as an antigen and activate immune responses. The resulting inflammatory mediators could in turn upregulate casein gene expression, creating a positive feedback loop that promotes inflammation and sensitization. In contrast, LPG appears to may pose a lower sensitization risk, owing to reduced casein gene expression and higher levels of anti-inflammatory factors. This study offers a cellular and molecular framework for understanding differences in the allergenicity of goat milk.

Although the sample size was limited (2 vs 3), this design is consistent with recent high-impact single-cell studies that have revealed meaningful biological insights despite similar cohort sizes (Becker et al., 2021; Zorc et al., 2024). Rigorous quality control, batch correction, and precise cell-type annotation contributed to data reliability. Nonetheless, several limitations remain. The small sample size restricts statistical power and generalizability. Potential cross-contamination—especially of highly expressed epithelial genes such as caseins—cannot be fully excluded despite stringent filtering. Individual variations and genetic backgrounds may also influence epithelial cell proportions. Future studies with larger sample sizes, validated cell-type-specific profiles, and integration of clinical and milk composition data are needed to confirm and extend these findings.

5. Conclusion

This study provides a preliminary single-cell transcriptome map of mid-lactation Saanen goat milk, showing significant variation in the proportion of EPCs (23.18 %–94.09 %), which is positively correlated with SCC. In samples with HPG, immune-cell-dominated differentiation trajectories were observed, alongside upregulation of pro-inflammatory and casein family genes (*CSN1S2*, *CSN2*, *CSN3*) in immune cells. Therefore, this study proposes that the EPCs proportion in goat milk could serve as a dynamic biomarker for monitoring mammary-gland health and milk safety. Future investigations are necessary to further elucidate the influence of EPC content on lactation and immune function. Additionally, the integration of scRNA-seq technologies into dairy research holds the potential to inform the development of precision-driven, health-promoting dairy products, thereby addressing the increasing consumer demand for both safety and enhanced nutritional value.

CRediT authorship contribution statement

Ren Tao: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology. **Cong He:** Supervision, Methodology, Investigation. **Liu Yaolong:** Visualization, Validation. **Liu Chengli:** Validation, Software, Formal analysis. **Ruan Pengcheng:** Software, Investigation. **Wang Zixuan:** Visualization, Validation. **Long Hao-yuan:** Software. **Simone Ceccobelli:** Writing – original draft. **Hu Pengfei:** Visualization. **Zeng Yan:** Formal analysis, Data curation. **Han Yanguo:** Software, Methodology. **Sun Yawang:** Methodology, Formal analysis. **E. Guang-xin:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2025.107872.

Data availability

Data will be made available on request.

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