

Changes of collagen content in lung tissues of plateau yak and its mechanism of adaptation to hypoxia

Jingyi Li¹, Nating Huang¹, Xun Zhang¹, Ci Sun¹, Jiarui Chen¹ and Qing Wei^{1,2}

¹ College of Eco-Environmental Engineering, Qinghai University, Xining, Qinghai, China

² State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining, Qinghai, China

ABSTRACT

Collagen is crucial for tissue structure, functional maintenance, and cellular processes such as proliferation and differentiation. However, the specific changes in collagen expression and its associated genes in the lung tissues of yaks at high altitudes and their relationship with environmental adaptation remain poorly understood. Studying differences in the content of collagen fibers and gene expression between yaks at high (4,500 m) and low (2,600 m) altitudes, as well as between cattle at low altitudes (2,600 m). Using Masson staining, we found that the collagen fiber content in the lung tissues of yaks at low altitude was significantly higher compared to yaks at high altitude and cattle at the same altitude ($P < 0.05$). It was revealed through transcriptomic analyses that genes differentially expressed between high and low altitude yaks, as well as between low altitude yaks and cattle, were notably enriched in pathways related to cell adhesion, collagen synthesis, focal adhesion, and ECM-receptor interactions. Specifically, genes involved in mesenchymal collagen synthesis (e.g., *COL1A1*, *COL1A2*, *COL3A1*), basement membrane collagen synthesis (e.g., *COL4A1*, *COL4A2*, *COL4A4*, *COL4A6*), and peripheral collagen synthesis (e.g., *COL5A1*, *COL6A1*, *COL6A2*, *COL6A3*) were significantly upregulated in the lung tissues of yaks at low altitude compared to their high altitude counterparts and cattle ($P < 0.05$). In conclusion, yaks at lower altitudes exhibit increased collagen synthesis by upregulating collagen gene expression, which contributes to maintaining alveolar stability and septal flexibility. Conversely, the expression of collagen genes in yak lung tissues was down-regulated with the increase in altitude, and it was speculated that the decrease in collagen may be used to constrain the function of elastic fibers that are more abundant at high altitude, so as to enable them to adapt to the harsh environment with hypoxia and high altitude. This adaptation mechanism highlights the role of collagen in environmental acclimatization and contributes to our understanding of how altitude and species influence collagen-related physiological processes in yaks.

Submitted 8 July 2024

Accepted 16 September 2024

Published 1 October 2024

Corresponding author

Qing Wei, xwq3519@sina.com

Academic editor

Viktor Brygadyrenko

Additional Information and
Declarations can be found on
page 14

DOI 10.7717/peerj.18250

© Copyright
2024 Li et al.

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

Subjects Biochemistry, Bioinformatics, Ecology, Zoology

Keywords Yak, Cattle, Lung tissue, Collagen, Plateau adaptation

INTRODUCTION

The yak (*Bos grunniens*) is a typical plateau mammal whose biological adaptations have developed over an extended period in this environment, resulting in unique and notable characteristics (Sapkota *et al.*, 2022). For example, yaks exhibit features such as efficient oxygen utilization (Wan *et al.*, 2021), well-developed lung structures (Ge *et al.*, 2021), adaptive hemoglobin regulation mechanisms (Xin *et al.*, 2019), and specialized skin and hair that provide effective cold tolerance (Krishnan *et al.*, 2018). The development and evolution of these biological traits are crucial for the survival and reproduction of yaks in highland areas. Additionally, they offer an ideal model for studying the biological mechanisms underlying animal adaptation to extreme environments.

Collagen is a crucial structural protein that serves as the fundamental unit for collagen fiber formation and is a major component of the extracellular matrix (ECM). Collagen interacts with various receptor families and cells to regulate their proliferation, migration, and differentiation. It also plays a key role in tissue connectivity, structural support, and the maintenance of physiological functions (Gordon & Hahn, 2010). Collagen regulation involves modulating its biosynthetic pathways through various signaling mechanisms and changes in gene expression (Angre *et al.*, 2022). As of now, 28 types of collagen have been identified through molecular biology techniques and gene cloning (Ricard-Blum, 2011). Type I collagen is the most widely distributed, performing various functions including regulating collagen fiber formation, repairing tissue damage, and supporting and protecting the integrity of tissues and organs (Baguisi *et al.*, 1997; Saito *et al.*, 2001; Chen *et al.*, 2019). Type I and Type III collagen are both involved in organizing collagen fibers and synthesizing collagenous materials (Ahtikoski *et al.*, 2001). Type IV collagen is widely expressed in all organs and is crucial for the construction of the basement membrane in blood vessels and other tissues. It maintains membrane integrity and supports tissue-specific functions (Pöschl *et al.*, 2004; Volonghi *et al.*, 2010; Mao *et al.*, 2015). Collagen gene expression has been shown to correlate with high-altitude acclimatization in animals (Wiener *et al.*, 2021). Changes in its expression contribute to angiogenesis and vascular remodeling (Minor & Coulombe, 2020), regulation of alveolar surface tension (Shi *et al.*, 2022), and ECM remodeling (Zhang *et al.*, 2011), thereby optimizing lung structure for adaptation to hypoxic environments. Reduced collagen content in mouse lungs restricts the progression of pulmonary hypertension (Schreier *et al.*, 2013; Chen *et al.*, 2006). However, the relationship between changes in collagen gene expression and environmental acclimatization in the lung tissues of plateau yaks remains unknown.

Therefore, this study aimed to investigate the relationship between collagen content and gene expression changes in the lung tissues of plateau yaks and their environmental adaptation.

MATERIALS AND METHODS

Experimental animals

This study selected three adult male yaks from Qumalai County (QML-Y, high-altitude yak at 4,500 m), three adult male yaks from Xunhua County (XH-Y, low-altitude yak at

2,600 m), and three adult male cattle from Xunhua County (XH-C, low-altitude yellow cattle at 2,600 m), totaling nine animals. At the same elevation, XH-C served as the control group while XH-Y was the experimental group. For different elevations within the same species, XH-Y was the control group and QML-Y was the experimental group. All animals were clinically healthy. The yaks were euthanized by exsanguination in a slaughterhouse. Lungs were harvested within 30 min of death, and deep tissue samples were collected from the diaphragmatic lobe of the left lung. Some tissues were snap-frozen in liquid nitrogen for long-term storage, while smaller samples were fixed in 4% paraformaldehyde for subsequent analysis. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Qinghai University, Xining, China (Approval No.: PJ-2023037). All methods adhered to the ARRIVE 2.0 guidelines and the Guidelines for Ethical Review of Animal Welfare in Laboratory Animals (GB/T 35892-2018) of the People's Republic of China. All local regulations and laws were adhered to. All cattle and yaks used in this study were purchased from local farmers in Qinghai Province, China.

Histologic staining

Paraffin sections of lung tissue

Fresh lung tissue specimens from both yak and cattle were excised and immediately fixed in 4% paraformaldehyde for at least 24 h. The tissues were then trimmed to a uniform thickness of 1–2 mm and placed into designated embedding cassettes. Residual fixative was meticulously washed off with running tap water, and the tissues were air-dried before proceeding with a graded dehydration series. Dehydration began with an overnight immersion in 70% ethanol, followed by immersion in 80% ethanol the next day. The tissues were then sequentially transferred through 90% ethanol, 95% ethanol, 100% ethanol I, and 100% ethanol II for gradient dehydration. After dehydration, the specimens were cleared with xylene and embedded in paraffin. The paraffin blocks were cooled on a –20 °C freezing table, sectioned into 4 µm slices, and set aside.

Masson staining

The paraffin sections were sequentially treated with xylene I for 8 min, xylene II for 8 min, anhydrous ethanol I for 6 min, anhydrous ethanol II for 6 min, 95% ethanol for 6 min, 85% ethanol for 6 min, and 75% ethanol for 5 min, followed by rinsing with running water. The sections were then stained with Weigert's Iron Hematoxylin Staining Solution for 5 min, washed thoroughly with water, and blued using Masson's bluing solution before another wash with water. Next, the sections were stained with Masson Lichun red acidic magenta solution for 5 min, rinsed with running water, treated with phosphomolybdic acid solution for 5 min, and then the excess liquid was removed. They were subsequently stained with aniline blue solution for 3 min, followed by rinsing with 1% glacial acetic acid aqueous solution until no blue color remained. Finally, the sections were briefly washed with 95% ethanol, dehydrated with anhydrous ethanol for 3 min, and cleared in xylene III for 5 min, xylene II for 5 min, and xylene I for 5 min before being mounted with neutral gum. Lung tissue sections were stained using the Masson method, resulting in blue-stained collagen fibers and red-stained cytoplasm. All staining solutions were prepared at identical

concentrations, and the staining times, temperatures, and washing procedures were standardized. All samples were fixed with the same fixative for an identical duration, and processed with consistent dehydration and clearing procedures. The embedding method was also uniform, ensuring that the tissue sections had the same thickness, thereby maintaining staining consistency across samples.

Observation and measurement

Stained tissue sections were observed and photographed with a Nikon optical microscope (DS-Ri2) and subsequently measured using Image-Pro Plus 6.0 software. The measurement data were analyzed using SPSS 26.0, with results presented as mean \pm standard deviation ($\bar{X} \pm SD$). A t-test was conducted to assess differences, utilizing SPSS 26.0.

Transcriptome sequencing and analysis

Lung tissue samples were collected from cattle and yaks at the same altitude, as well as from yaks at different altitudes. RNA was extracted using the Trizol method, and mRNA was enriched with Oligo (dT) magnetic beads to construct cDNA libraries. The transcriptome was sequenced using the Illumina HiSeq 2500 platform. Raw data were filtered, and the clean reads were compared to the reference genome for gene annotation, quantitative analysis, and differential expression analysis. In the gene analysis section, we also performed analyses for gene coverage, sequencing randomness, and sequencing saturation. Gene coverage represents the percentage of bases in a gene that are covered by reads, calculated as the ratio of the bases covered by reads to the total bases in the gene's coding region. To evaluate the randomness of mRNA interruption, we analyzed the distribution of reads across reference genes. Due to variations in the lengths of different reference genes, we normalized read positions to relative positions (the ratio of read position to gene length * 100) and counted reads across different positional ratios. Ideally, with good randomness, reads should be evenly distributed across gene sites. Sequencing saturation analysis assesses whether the sequencing depth of a sample is sufficient to detect most genes. As the sequencing depth (number of reads) increases, the number of detected genes rises until reaching a plateau, indicating saturation in gene detection. The input data for gene differential expression analysis consisted of read count data from gene expression level assessments. The differential expression analysis was performed using DESeq2 and was divided into three main steps: normalization of read counts, calculation of *P*-values based on the model, and correction for multiple hypothesis testing to obtain the false discovery rate (FDR). Genes with $|\log_2(\text{FoldChange})| > 0.585$ and $\text{FDR} < 0.05$ were identified as significant, based on variance analysis results. This set of significant genes was then clustered and analyzed.

To ensure the reliability and reproducibility of the experimental results, this study employed both biological and technical replicates. For biological replication, nine independent samples were collected from local herders and divided into three groups, with each group undergoing three replicates under identical conditions. Technical replication involved conducting all experiments under stringent quality control, maintaining

Table 1 Gene primer sequence information.

Gene name	Primer types	Sequence (5'-3')
COL1A1	Upstream primers	TCCTTCTGGTCCTCGTGGTCTC
	Downstream primers	TCGCCATCATCTCCGTTCTT
COL1A2	Upstream primers	GCCACCCAGAATGGAGCAGT
	Downstream primers	GATGCAGGTTTCGCCAGTAGA
COL6A2	Upstream primers	CGGGTGCTGTGACTGTGAGAA
	Downstream primers	CAGCCTGTTGACCACGTTGAT
GAPDH	Upstream primers	GGTCACCAGGGCTGCTTTTA
	Downstream primers	CCAGCATCACCCCACTTGAT

consistent temperature, time, and reagent lot number. The statistical power of this experimental design, calculated in RNASeqPower is 0.95 (https://rodrigo-arcoverde.shinyapps.io/rnaseq_power_calc/). This result illustrates the validity and rigor of our transcriptome sequencing.

Fluorescence quantitative PCR

Real-time quantitative PCR was performed using cDNAs from lung tissues of yak and cattle collected from high and low altitudes. Relative mRNA levels were measured using 7.5 μ l of 2 \times Universal Blue SYBR Green qPCR Master Mix, 1.5 μ l of 2.5 μ M gene primers (both upstream and downstream), 2.0 μ l of the reverse transcription product (cDNA), and 4.0 μ l of nuclease-free water. *GAPDH* served as the internal reference gene, with the primer sequences listed in Table 1. The mRNA expression of *COL1A1*, *COL1A2*, and *COL6A2* genes in lung tissues was analyzed using the $2^{-\Delta\Delta CT}$ method for relative quantification. An analysis of variance was conducted using SPSS 26.0 software to identify significant differences.

RESULTS

Masson staining results of yak and cattle lung tissues

The blue-stained regions of the lung tissue sections revealed collagen fiber structures. Collagen fibers were more abundant in the lung tissues of yaks at low altitude compared to both cattle at low altitude and yaks at high altitude (Fig. 1A). Quantitative analysis revealed that the percentage of collagen fibers in the lung tissues of yaks at low altitude was significantly higher than in cattle ($P = 0.000$) (Fig. 1B). Additionally, the percentage of collagen fibers in the lung tissues of yaks at high altitude was significantly lower than in yaks at low altitude ($P = 0.008$) (Fig. 1C).

Overall statistics of differential genes between groups

The results revealed 3,684 differentially expressed genes in yaks compared to cattle in low-altitude areas, including 2,189 up-regulated genes and 1,495 down-regulated genes (Fig. 2A). Additionally, there were 2,326 differentially expressed genes when comparing

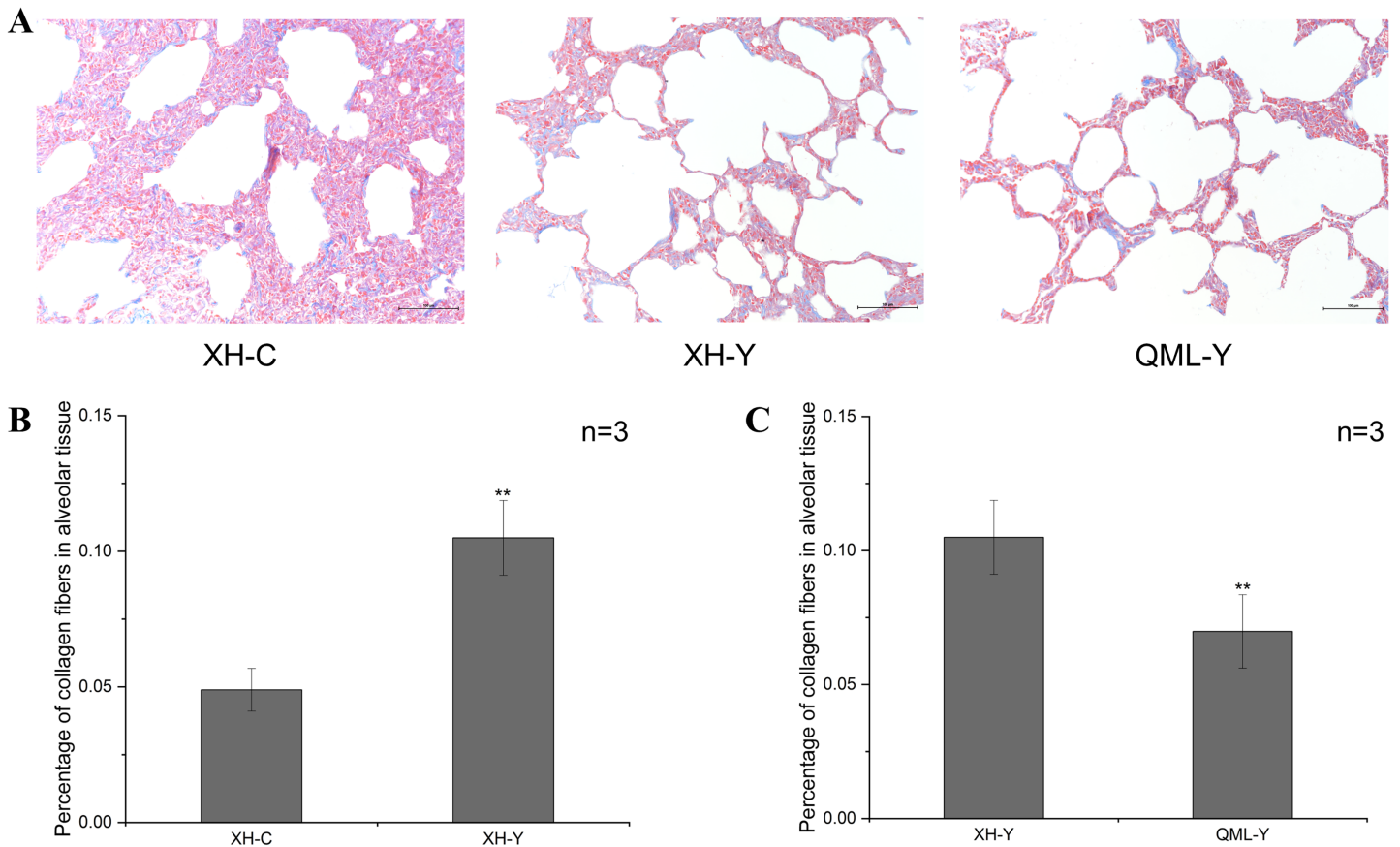


Figure 1 Masson staining of yak and cattle lung tissue. (A) Masson staining of XH-C, XH-Y and QML-Y. (B) Percentage of collagen fibers in XH-Y vs XH-C lung tissues. (C) Percentage of collagen fibers in QML-Y vs XH-Y lung tissues. Asterisks (**) denote a highly significant difference, $P < 0.01$.

Full-size [DOI: 10.7717/peerj.18250/fig-1](https://doi.org/10.7717/peerj.18250/fig-1)

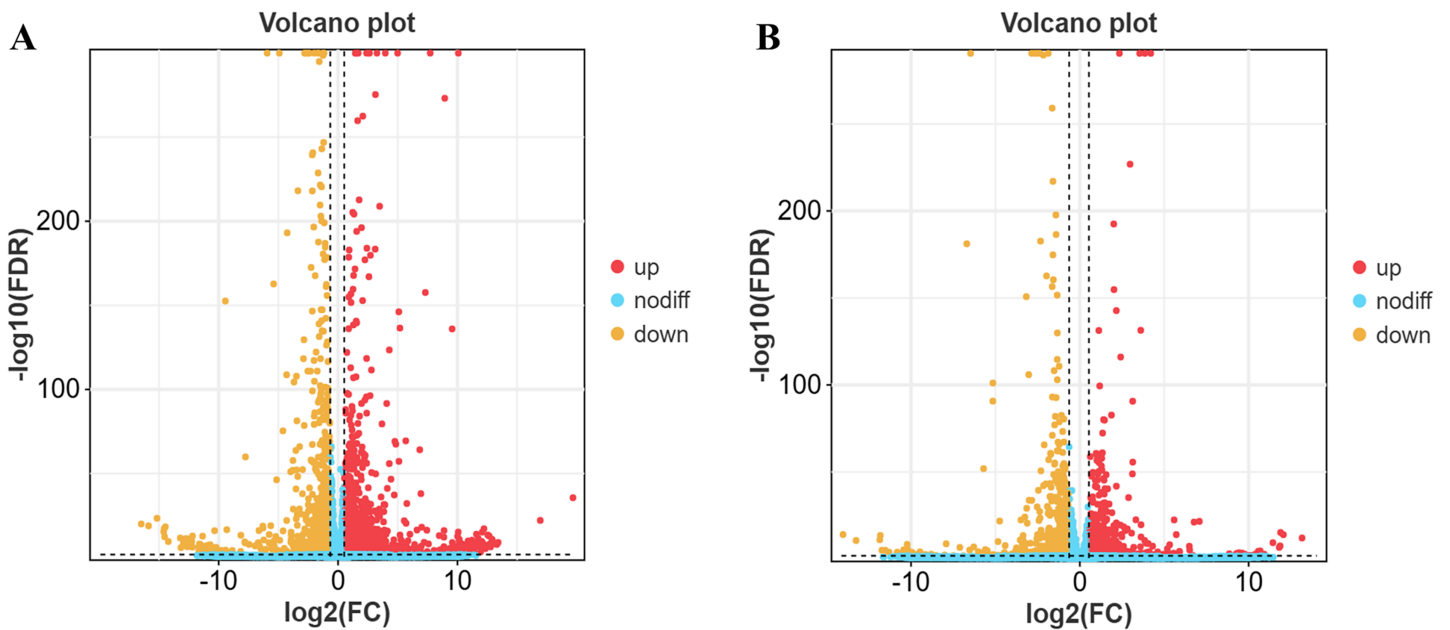


Figure 2 Differential gene volcano map. (A) XH-Y vs XH-C. (B) QML-Y vs XH-Y.

Full-size [DOI: 10.7717/peerj.18250/fig-2](https://doi.org/10.7717/peerj.18250/fig-2)

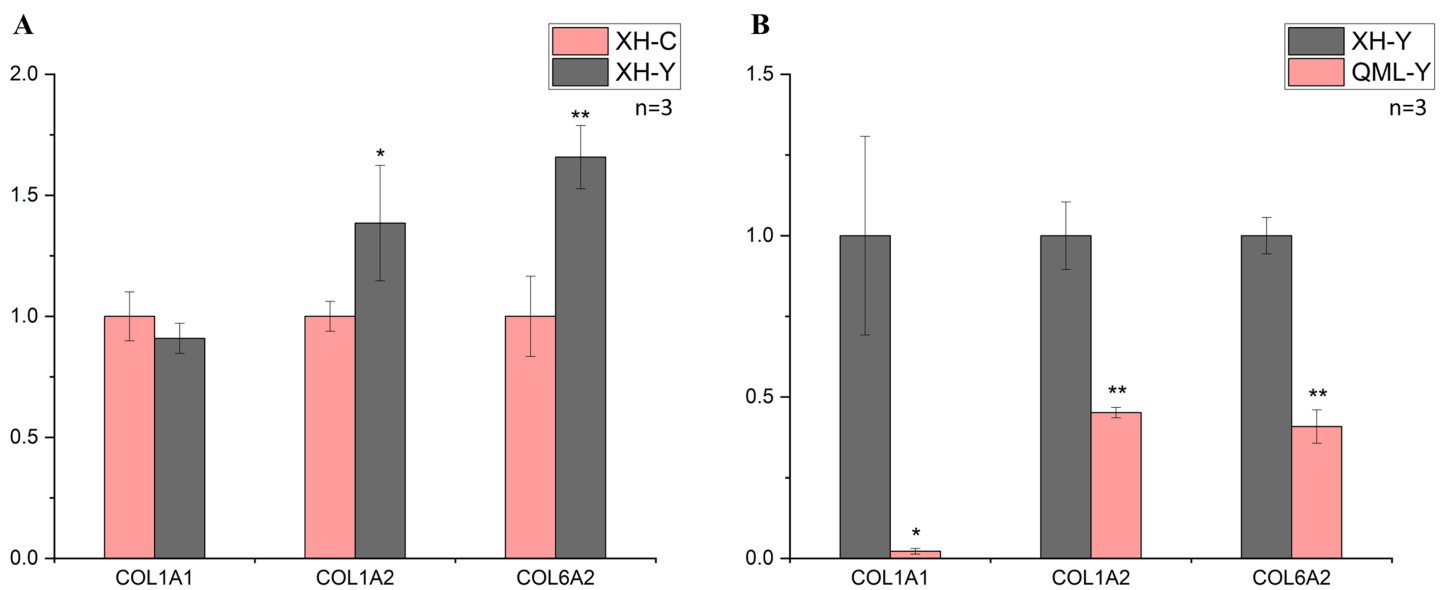


Figure 3 Changes in mRNA expression levels of collagen-related genes. (A) XH-Y vs XH-C. (B) QML-Y vs XH-Y. An asterisk (*) denotes significant difference, $P < 0.05$. Two asterisks (**) denote highly significant difference, $P < 0.01$. [Full-size !\[\]\(fd7fe780e8fd8eece60268c87d0c3e04_img.jpg\) DOI: 10.7717/peerj.18250/fig-3](https://doi.org/10.7717/peerj.18250/fig-3)

yaks from high and low-altitude areas, with 1,340 up-regulated genes and 986 down-regulated genes (Fig. 2B).

Fluorescence quantitative PCR validation analysis

In the low-altitude comparison between yaks and cattle, relative quantitative analysis was performed on the *COL1A1*, *COL1A2* and *COL6A2* genes using cattle as the standard (Fig. 3A). The *COL1A1* gene showed no significant differential expression between the two groups ($P = 0.247$), while the *COL1A2* gene exhibited significant differential expression ($P = 0.049$), and the *COL6A2* gene displayed highly significant differential expression ($P = 0.006$).

In the comparison between high-altitude and low-altitude yaks, the *COL1A1*, *COL1A2* and *COL6A2* genes were analyzed using relative quantitative methods with low-altitude yaks as the reference (Fig. 3B). The *COL1A1* gene showed significant differential expression between the two groups ($P = 0.029$), while the *COL1A2* ($P = 0.001$) and *COL6A2* ($P = 0.000$) genes exhibited highly significant differential expression. The quantitative detection results were consistent with the transcriptome sequencing trends.

Gene ontology enrichment analysis

Gene Ontology (GO) analysis revealed that low-altitude yaks were enriched in biological processes such as bioadhesion, cell adhesion, and extracellular matrix organization, as well as cellular components including cell periphery, extracellular matrix, and collagen-containing extracellular matrix, compared to low-altitude cattle (Fig. 4A); and high-altitude yaks were enriched in similar biological processes and cellular components compared to low-altitude yaks (Fig. 4B). These findings indicate that differentially expressed genes in lung tissues are involved in cell adhesion and collagen synthesis in both groups.

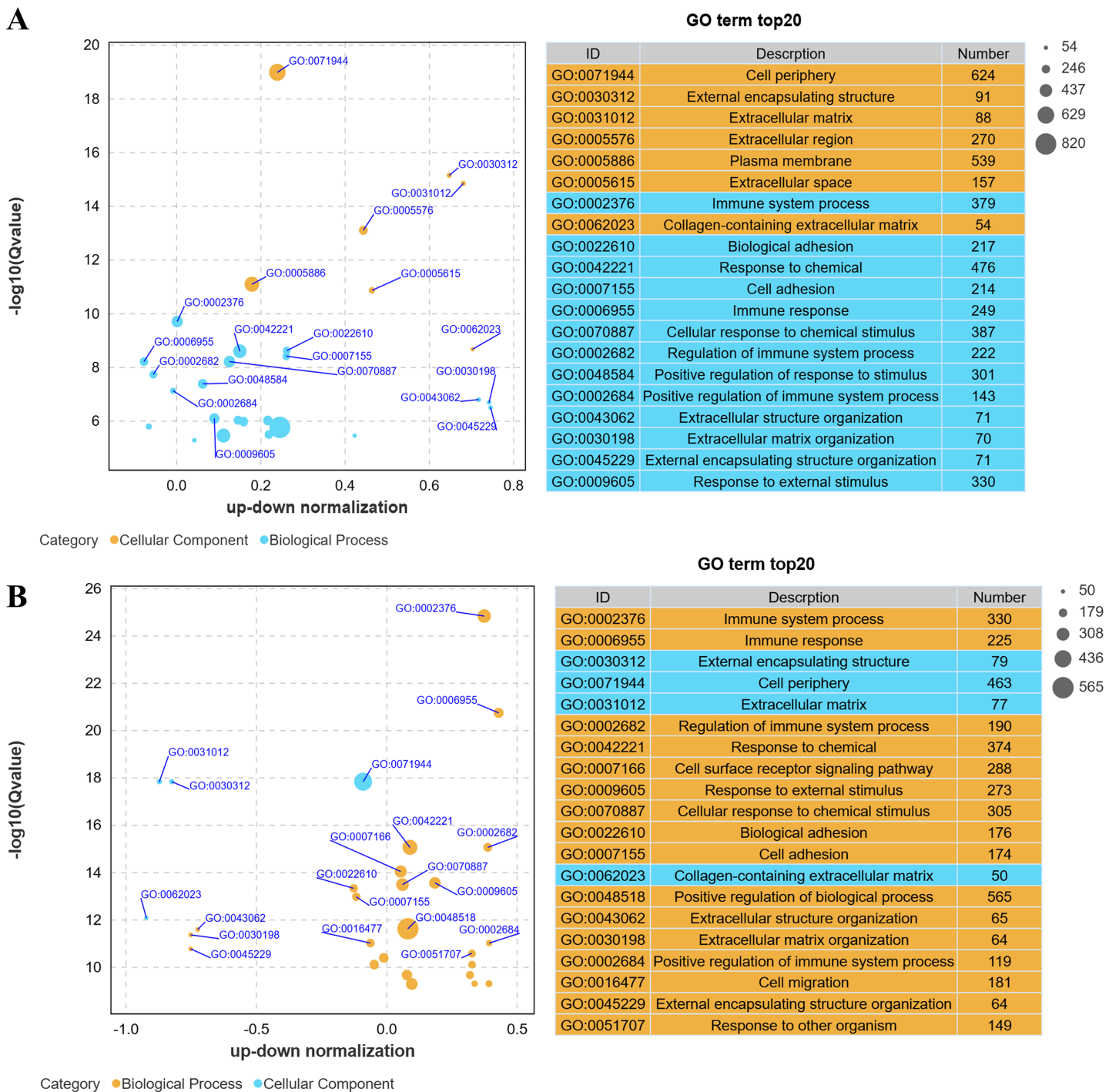


Figure 4 Bubble plot of GO enrichment differences. (A) XH-Y vs XH-C. (B) QML-Y vs XH-Y. [Full-size DOI: 10.7717/peerj.18250/fig-4](https://doi.org/10.7717/peerj.18250/fig-4)

Kyoto encyclopedia of genes and genomes enrichment analysis

Differentially expressed genes in the lung tissues of yaks and cattle at low altitude were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Analysis of the top 20 pathways with the most significant differences (smallest Q-value)

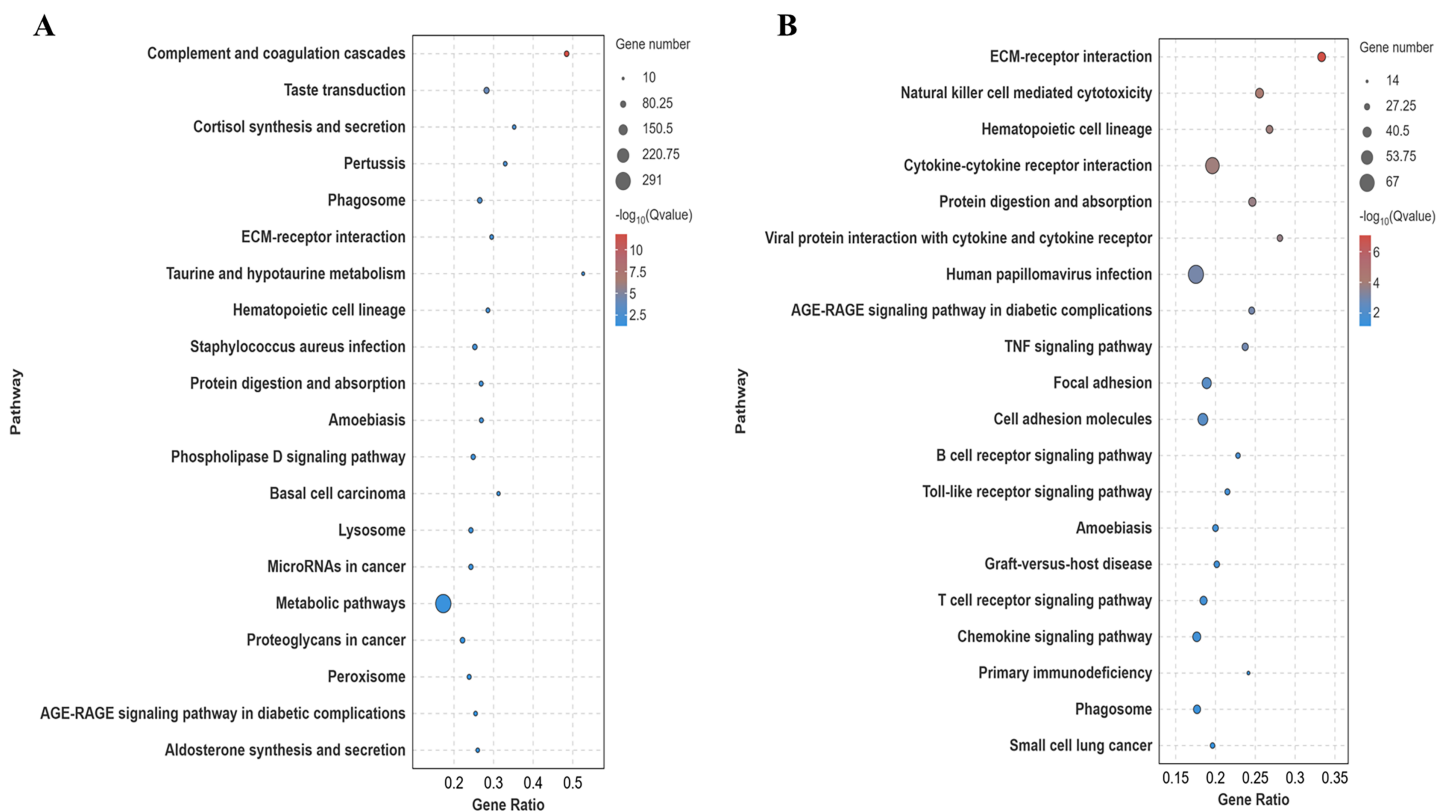


Figure 5 KEGG significance bubble plot. (A) XH-Y vs XH-C. (B) QML-Y vs XH-Y.

Full-size DOI: [10.7717/peerj.18250/fig-5](https://doi.org/10.7717/peerj.18250/fig-5)

revealed that these genes were enriched in signaling pathways such as ECM-receptor interaction and protein digestion and absorption (Fig. 5A). Similarly, differentially expressed genes in yak lung tissues from both high and low altitude regions were annotated using the KEGG database. Analysis of the top 20 pathways with the most significant differences (smallest Q-value) showed enrichment in signaling pathways including ECM-receptor interaction, protein digestion and uptake, focal adhesion, and cell adhesion molecules (Fig. 5B). These results suggest that the differentially expressed genes in the lung tissues of yaks and cattle at low altitude, as well as yaks at both high and low altitudes, are enriched in pathways related to collagen synthesis.

Screening of differentially expressed collagen genes

Based on existing studies and GO annotation, 19 significantly different collagen genes were identified in the lung tissues of yak and cattle at low altitude. Among these, 18 genes were up-regulated and one gene was down-regulated in yak lung tissues (Table 2). Additionally, 23 significantly different collagen genes were found in yak lung tissues at both high and low altitudes, with all of these genes being down-regulated at high altitude (Table 3).

Significant differential gene expression changes in collagen

The differentially expressed collagen genes in the two groups were categorized into three types: mesenchymal collagen synthesis-related genes, basement membrane collagen

Table 2 Annotation of XH-Y vs XH-C significantly different collagen-related genes.

ID	Symbol	Description
ENSBGRG00000005548	COL1A1	XP_006041214.2 collagen alpha-1 (I) chain isoform X1
ENSBGRG00000013466	COL1A2	Collagen type I alpha 2 chain
ENSBGRG00000003247	COL3A1	Collagen type III alpha 1 chain
ENSBGRG00000004437	COL4A1	Collagen type IV alpha 1 chain
ENSBGRG00000005083	COL4A2	Collagen type IV alpha 2 chain
ENSBGRG00000012460	COL4A4	Collagen type IV alpha 4 chain
ENSBGRG00000021026	COL4A6	Collagen type IV alpha 6 chain
ENSBGRG00000005255	COL5A1	Collagen type V alpha 1 chain
ENSBGRG00000000813	COL6A1	Collagen type VI alpha 1 chain
ENSBGRG00000000863	COL6A2	Collagen type VI alpha 2 chain
ENSBGRG00000017439	COL6A3	Collagen alpha-3(VI) chain-like
ENSBGRG00000003956	COL6A5	Collagen type VI alpha 5 chain
ENSBGRG00000016164	COL14A1	Collagen type XIV alpha 1 chain
ENSBGRG00000020183	COL15A1	Collagen type XV alpha 1 chain
ENSBGRG00000008612	COL16A1	Collagen type XVI alpha 1 chain
ENSBGRG00000024606	COL21A1	Collagen type XXI alpha 1 chain
ENSBGRG00000011762	COL23A1	Collagen type XXIII alpha 1 chain
ENSBGRG00000025649	COL26A1	Collagen type XXVI alpha 1 chain
ENSBGRG00000007346	COL28A1	Collagen type XXVIII alpha 1 chain

Table 3 Annotation of QML-Y vs XH-Y significantly different collagen-related genes.

ID	Symbol	Description
ENSBGRG00000005548	COL1A1	XP_006041214.2 collagen alpha-1(I) chain isoform X1
ENSBGRG00000013466	COL1A2	Collagen type I alpha 2 chain
ENSBGRG00000024422	COL2A1	Collagen type II alpha 1 chain
ENSBGRG00000003247	COL3A1	Collagen type III alpha 1 chain
ENSBGRG00000004437	COL4A1	Collagen type IV alpha 1 chain
ENSBGRG00000005083	COL4A2	Collagen type IV alpha 2 chain
ENSBGRG00000012460	COL4A4	Collagen type IV alpha 4 chain
ENSBGRG00000021853	COL4A5	Collagen type IV alpha 5 chain
ENSBGRG00000021026	COL4A6	Collagen type IV alpha 6 chain
ENSBGRG00000005255	COL5A1	Collagen type V alpha 1 chain
ENSBGRG00000003463	COL5A2	Collagen type V alpha 2 chain
ENSBGRG00000001716	COL5A3	Collagen type V alpha 3 chain
ENSBGRG00000000813	COL6A1	Collagen type VI alpha 1 chain
ENSBGRG00000000863	COL6A2	Collagen type VI alpha 2 chain
ENSBGRG00000017439	COL6A3	Collagen alpha-3(VI) chain-like
ENSBGRG00000002584	COL8A1	Collagen type VIII alpha 1 chain

Table 3 (continued)

ID	Symbol	Description
ENSBGRG00000017451	COL9A2	Collagen type IX alpha 2 chain
ENSBGRG00000006189	COL12A1	Collagen type XII alpha 1 chain
ENSBGRG00000020183	COL15A1	Collagen type XV alpha 1 chain
ENSBGRG00000008612	COL16A1	Collagen type XVI alpha 1 chain
MSTRG.1779	COL18A1	XP_024830811.1 collagen alpha-1(XVIII) chain isoform X2 [Bos taurus]
ENSBGRG00000024606	COL21A1	Collagen type XXI alpha 1 chain
ENSBGRG00000025649	COL26A1	Collagen type XXVI alpha 1 chain

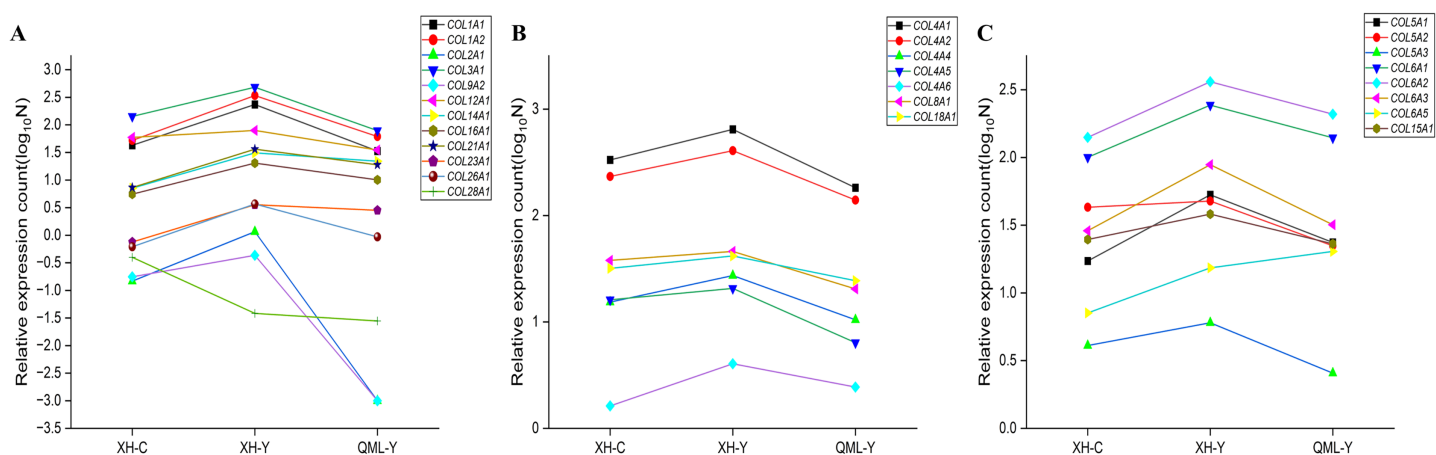


Figure 6 Significant differential gene expression level changes in collagen. (A) Genes related to mesenchymal collagen synthesis. (B) Genes related to basement membrane collagen synthesis. (C) Genes related to peripheral cellular collagen synthesis.

Full-size DOI: 10.7717/peerj.18250/fig-6

synthesis-related genes, and peripheral cellular collagen synthesis-related genes. Specific values of relative gene expression are presented in [Supplemental Material 1](#). Among these, interstitial collagen synthesis-related genes such as *COL1A1*, *COL1A2*, *COL2A1*, *COL3A1*, *COL9A2*, *COL12A1*, *COL14A1*, *COL16A1*, *COL21A1*, *COL23A1*, and *COL26A1* were highly expressed in the lung tissues of yak from low-altitude areas compared to both cattle from low-altitude areas and yak from high-altitude areas (Fig. 6A). Basement membrane collagen synthesis-related genes, including *COL4A1*, *COL4A2*, *COL4A4*, *COL4A5*, *COL4A6*, *COL8A1*, and *COL18A1* were also highly expressed in the lung tissues of yak from low-altitude areas compared to cattle from low-altitude areas and yak from high-altitude areas (Fig. 6B). Peripheral collagen synthesis-related genes such as *COL5A1*, *COL5A2*, *COL5A3*, *COL6A1*, *COL6A2*, *COL6A3*, and *COL15A1* were highly expressed in the lung tissues of yak from low-altitude areas compared to cattle from low-altitude areas and yak from high-altitude areas. In contrast, *COL6A5* was more highly expressed in the lung tissues of yak from high-altitude areas compared to those from low-altitude areas and cattle (Fig. 6C).

DISCUSSION

Lung tissue is the primary organ for oxygen entry into the body, with the alveoli serving as the main site for gas exchange and the functional unit of lung tissue (Warburton, 2021). As a hypoxia-sensitive organ, the structure of lung tissue varies among species at the same altitude and within the same species at different altitudes to meet the organism's oxygen demands (Jing et al., 2022). GO functional analysis indicated that differentially expressed genes in the lung tissues of yaks at high and low altitudes, as well as between cattle at low altitudes, are involved in processes like cell adhesion and collagen synthesis. KEGG analysis further demonstrated that differentially expressed genes in lung tissues of yaks and cattle at low altitude, as well as yaks at high and low altitudes, were enriched in pathways related to adhesion (e.g., focal adhesion and cell adhesion molecules) and collagen synthesis (e.g., ECM-receptor interactions and protein digestion and uptake). The extracellular matrix (ECM), composed of the basement membrane (BM) and intercellular matrix, encompasses structures like collagen and elastic fibers (Kular, Basu & Sharma, 2014). The ECM plays a crucial role in tissue and organ morphogenesis, and in maintaining cellular and tissue structure and function (Krieg & Aumailley, 2011). Cellular adhesion serves as a key mechanism for cells to connect to the ECM, with collagen playing a pivotal role in this process (Mishra & Manavathi, 2021; Yamaguchi & Knaut, 2022). Based on this, we hypothesize that plateau yaks regulate collagen and elastin synthesis, thereby influencing the cell adhesion process. This, in turn, regulates lung tissue function.

Zhou et al. (2015) observed that small pulmonary arterioles in the lungs of adult yellow cattle contained fewer elastic fibers compared to similar-sized arterioles in adult yaks. Elastic fibers confer elasticity to vessel walls, while collagen fibers provide tensile strength (Zulliger et al., 2004; Kumar et al., 2013). Additionally, elastic fibers help maintain collagen fiber conformation, and their combination enhances the resistance of the vessel walls to compression (Mizuno et al., 2005). Research has shown that alveolar septa in plateau yaks are significantly thicker than those in plains cattle (Zhang et al., 2023). The presence of collagen fibers in these septa contributes to their flexibility, which is crucial for lung tissue function (Mascaretti et al., 2009). Meanwhile, the present study found that collagen fibers were more abundant in the lung tissue of yaks at low altitudes compared to cattle, and the expression of collagen synthesis-related genes was also higher in yaks. Among these genes, *COL3A1*, which is involved in collagen synthesis in mesenchymal tissues, is crucial for maintaining the morphology and structure of the skin, tissues, and organs. Up-regulation of *COL3A1* in the dense layer of sheep fetal membranes has been shown to enhance membrane tensile strength (Mi et al., 2018). Additionally, *COL3A1* facilitates cell adhesion, migration, proliferation, and differentiation through interactions with integrins, which are cell surface receptors (Kim et al., 2005; Kuivaniemi & Tromp, 2019). Additionally, the peripheral collagen synthesis-related genes *COL6A1* and *COL6A2* are crucial for supporting tissue structure, maintaining extracellular matrix elasticity, and participating in cellular signaling (Fitzgerald et al., 2008). They also interact with other collagens and basement membranes to reinforce the extracellular matrix network (Bonaldo et al., 1990). The interstitium supports the alveolar network through collagen, regulates intercellular

adhesion, and is critical for maintaining lung tissue integrity (Baldock et al., 2003; Atkinson et al., 2008; Voiles et al., 2014). Yak lung tissue, which is rich in collagen fibers, exhibits greater resistance to compression and enhanced flexibility compared to cattle lung tissue. Additionally, the basement membrane of yak lung tissue is more structurally stable and functionally superior to that of cattle, featuring increased extracellular matrix elasticity, a more extensive extracellular matrix network, and stronger interactions with other molecules.

Studies have demonstrated that lung tissues of high-altitude indigenous animals, including Tibetan sheep, plateau pika, plateau zokor, and Himalayan dry otter, contain more elastin fibers (Zhao et al., 2022; West et al., 2021). Specifically, yak lungs at high altitudes have a higher concentration of elastin fibers compared to those at lower altitudes, which helps ensure adequate ventilation (Li et al., 2021). However, excessive elastin deposition can impair ventilation and gas exchange, leading to reduced lung compliance (Yombo et al., 2023). In a healthy lung, a balance between collagen and elastin fibers is maintained, providing both structural strength to resist pressure and sufficient elasticity to accommodate respiratory movements (Halsey et al., 2023). Conversely, collagen fibers are highly resistant to tensile forces and may constrain the elastic function of elastin fibers (Kim et al., 2015; Rabkin, 2017). Abnormal deposition of collagen and extracellular matrix (ECM) components in lung tissue is a hallmark of pulmonary fibrosis (Quesnel et al., 2010). Chen et al. (2023) also found that Collagen I and Collagen III proteins were significantly increased in yak lung fibrotic tissues, indicating that collagen-related genes play a crucial role in the fibrosis process in yak lung tissues. In the present study, we observed that collagen fibers were more abundant in the lung tissues of yaks at low altitude compared to those at high altitude. Additionally, the expression of collagen synthesis-related genes was higher in the lung tissues of yaks at low altitude. Notably, the down-regulation of *COL1A1*, a gene involved in interstitial collagen synthesis, exhibited an anti-fibrotic effect (Niu et al., 2022). Gong et al. (2023) found that inhibiting *COL1A1* expression reduced the severity of pulmonary fibrosis induced in mice. Basement membrane collagen synthesis-related genes primarily include type IV collagen, such as *COL4A1*, *COL4A2*, *COL4A4*, and *COL4A6* (Yurchenco, 2011), with *COL4A1* also being closely linked to cell proliferation (Jin et al., 2017). Peripheral collagen synthesis genes, such as *COL5A1* and *COL5A2*, are often found alongside other collagen types. These genes contribute to the structural framework of tissues, extracellular matrix assembly, and cell signaling (Koch et al., 2001). Alveolar hypoxia, prevalent in non-high-altitude-adapted species, can contribute to the onset of pulmonary diseases such as pulmonary hypertension and fibrosis (Sydykov et al., 2021; Schoene, 1999). Pulmonary artery compliance decreases while collagen deposition increases in the context of pulmonary hypertension (Thenappan et al., 2016). Reduced collagen accumulation in mice has been shown to aid in preserving near-normal compliance and vascular resistance properties of the pulmonary circulation under hypoxic conditions, thereby mitigating the onset of pulmonary hypertension (Chen et al., 2006). Pulmonary fibrosis can be significantly improved through the inhibition of myofibroblast differentiation and collagen accumulation in rats

(*Andugulapati et al., 2020*). Therefore, reducing collagen fiber content in yak lung tissues at high altitudes may help mitigate the excessive accumulation of fibers due to elevated elastic fiber content. Lower expression of interstitial collagen synthesis-related genes helps reduce excessive fiber accumulation in lung tissues caused by the low-oxygen plateau environment, thus better protecting yak lung tissues and aiding adaptation to the harsh plateau conditions. The small sample size due to logistical challenges, so only the available samples were comparatively analyzed in relation to other animals in hypoxic environments. Future work will involve a comprehensive analysis of different fiber types in lung tissues to elucidate their relationships and to explain how structural and compositional changes in fibers impact the adaptation of plateau yaks to their environment.

CONCLUSIONS

Differentially expressed genes in the lung tissues of yak and cattle at low altitude, as well as yak at both high and low altitudes, are enriched in pathways and functions related to collagen synthesis. At the same altitude, yaks can increase collagen content by upregulating collagen-related genes, compared to cattle. This upregulation helps maintain the stability of alveolar structures, the flexibility of alveolar septa, and the integrity of the basement membrane in yaks. As altitude increases, collagen genes may be downregulated to decrease collagen content in yak lung tissues. This reduction helps limit the elasticity of elastic fibers, mitigates the fibrotic response in lung tissues, and aids in adaptation to the harsh hypoxic environment.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This work was supported by the Qinghai Basic Research Project (grant number: 2023-ZJ-708). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Qinghai Basic Research Project: 2023-ZJ-708.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Jingyi Li conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Nating Huang performed the experiments, prepared figures and/or tables, and approved the final draft.

- Xun Zhang analyzed the data, prepared figures and/or tables, and approved the final draft.
- Ci Sun analyzed the data, prepared figures and/or tables, and approved the final draft.
- Jiarui Chen conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Qing Wei conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (*i.e.*, approving body and any reference numbers):

This study was approved by the Institutional Animal Care and Use Committee of Qinghai University (Xining, China).

Data Availability

The following information was supplied regarding data availability:

The sequence data is available at NCBI SRA: [PRJNA1127767](https://www.ncbi.nlm.nih.gov/sra/PRJNA1127767).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.18250#supplemental-information>.

REFERENCES

- Ahtikoski AM, Koskinen SO, Virtanen P, Kovanen V, Takala TE. 2001.** Regulation of synthesis of fibrillar collagens in rat skeletal muscle during immobilization in shortened and lengthened positions. *Acta Physiologica Scandinavica* **172**(2):131–140
DOI [10.1046/j.1365-201X.2001.00848.x](https://doi.org/10.1046/j.1365-201X.2001.00848.x).
- Andugulapati SB, Gourishetti K, Tirunavalli SK, Shaikh TB, Sistla R. 2020.** Biochanin-a ameliorates pulmonary fibrosis by suppressing the TGF- β mediated EMT, myofibroblasts differentiation and collagen deposition in in vitro and in vivo systems. *Phytomedicine* **78**:153298
DOI [10.1016/j.phymed.2020.153298](https://doi.org/10.1016/j.phymed.2020.153298).
- Angre T, Kumar A, Singh AK, Thareja S, Kumar P. 2022.** Role of collagen regulators in cancer treatment: a comprehensive review. *Anti-Cancer Agents in Medicinal Chemistry* **22**(17):2956–2984 DOI [10.2174/1871520622666220501162351](https://doi.org/10.2174/1871520622666220501162351).
- Atkinson JJ, Adair-Kirk TL, Kelley DG, Demello D, Senior RM. 2008.** Clara cell adhesion and migration to extracellular matrix. *Respiratory Research* **9**(1):1 DOI [10.1186/1465-9921-9-1](https://doi.org/10.1186/1465-9921-9-1).
- Baguisi A, Arav A, Crosby TF, Roche JF, Boland MP. 1997.** Hypothermic storage of sheep embryos with antifreeze proteins: development in vitro and in vivo. *Theriogenology* **48**(6):1017–1024 DOI [10.1016/S0093-691X\(97\)00328-2](https://doi.org/10.1016/S0093-691X(97)00328-2).
- Baldock C, Sherratt MJ, Shuttleworth CA, Kielty CM. 2003.** The supramolecular organization of collagen VI microfibrils. *Journal of Molecular Biology* **330**(2):297–307
DOI [10.1016/S0022-2836\(03\)00585-0](https://doi.org/10.1016/S0022-2836(03)00585-0).
- Bonaldo P, Russo V, Bucciotti F, Doliana R, Colombatti A. 1990.** Structural and functional features of the alpha 3 chain indicate a bridging role for chicken collagen VI in connective tissues. *Biochemistry* **29**(5):1245–1254 DOI [10.1021/bi00457a021](https://doi.org/10.1021/bi00457a021).

- Chen P, He ZF, Wang F, Xie JP, He HH. 2023.** Expression study of Collagen1 and Collagen3 in lung fibrosis tissues of yak. *Journal of Nuclear Agricultural Sciences (China)* **37(6)**:1158–1165 DOI [10.11869/j.issn.1000-8551.2023.06.1158](https://doi.org/10.11869/j.issn.1000-8551.2023.06.1158).
- Chen X, Yang Y, Chang T, Xu B, Wei H. 2019.** Comparative transcriptome analysis of unusual localized skin laxity in Sika Deer (*Cervus nippon*). *DNA and Cell Biology* **38(7)**:670–677 DOI [10.1089/dna.2019.4730](https://doi.org/10.1089/dna.2019.4730).
- Chen YF, Feng JA, Li P, Xing D, Zhang Y, Serra R, Ambalavanan N, Majid-Hassan E, Oparil S. 2006.** Dominant negative mutation of the TGF-beta receptor blocks hypoxia-induced pulmonary vascular remodeling. *Journal of Applied Physiology* **100(2)**:564–571 DOI [10.1152/jappphysiol.00595.2005](https://doi.org/10.1152/jappphysiol.00595.2005).
- Fitzgerald J, Rich C, Zhou FH, Hansen U. 2008.** Three novel collagen VI chains, alpha4(VI), alpha5(VI), and alpha6(VI). *Journal of Biological Chemistry* **283(29)**:20170–20180 DOI [10.1074/jbc.M710139200](https://doi.org/10.1074/jbc.M710139200).
- Ge Q, Guo Y, Zheng W, Zhao S, Cai Y, Qi X. 2021.** Molecular mechanisms detected in yak lung tissue via transcriptome-wide analysis provide insights into adaptation to high altitudes. *Scientific Reports* **11(1)**:7786 DOI [10.1038/s41598-021-87420-7](https://doi.org/10.1038/s41598-021-87420-7).
- Gong H, Lyu X, Liu Y, Peng N, Tan S, Dong L, Zhang X. 2023.** Eupatilin inhibits pulmonary fibrosis by activating Sestrin2/PI3K/Akt/mTOR dependent autophagy pathway. *Life Sciences* **334(1)**:122218 DOI [10.1016/j.lfs.2023.122218](https://doi.org/10.1016/j.lfs.2023.122218).
- Gordon MK, Hahn RA. 2010.** Collagens. *Cell and Tissue Research* **339(1)**:247–257 DOI [10.1007/s00441-009-0844-4](https://doi.org/10.1007/s00441-009-0844-4).
- Halsey G, Sinha D, Dhital S, Wang X, Vyavahare N. 2023.** Role of elastic fiber degradation in disease pathogenesis. *Biochimica et Biophysica Acta—Molecular Basis of Disease* **1869(5)**:166706 DOI [10.1016/j.bbadis.2023.166706](https://doi.org/10.1016/j.bbadis.2023.166706).
- Jing X, Ding L, Zhou J, Huang X, Degen A, Long R. 2022.** The adaptive strategies of yaks to live in the Asian highlands. *Animal Nutrition* **9**:249–258 DOI [10.1016/j.aninu.2022.02.002](https://doi.org/10.1016/j.aninu.2022.02.002).
- Jin R, Shen J, Zhang T, Liu Q, Liao C, Ma H, Li S, Yu Z. 2017.** The highly expressed COL4A1 genes contributes to the proliferation and migration of the invasive ductal carcinomas. *Oncotargets and Therapy* **8(35)**:58172–58183 DOI [10.18632/oncotarget.17345](https://doi.org/10.18632/oncotarget.17345).
- Kim JK, Xu Y, Xu X, Keene DR, Gurusiddappa S, Liang X, Wary KK, Höök M. 2005.** A novel binding site in collagen type III for integrins alpha1beta1 and alpha2beta1. *Journal of Biological Chemistry* **280(37)**:32512–32520 DOI [10.1074/jbc.M502431200](https://doi.org/10.1074/jbc.M502431200).
- Kim J, Procknow JD, Yanagisawa H, Wagenseil JE. 2015.** Differences in genetic signaling, and not mechanical properties of the wall, are linked to ascending aortic aneurysms in fibulin-4 knockout mice. *American Journal of Physiology-Heart and Circulatory Physiology* **309(1)**:H103–H113 DOI [10.1152/ajpheart.00178.2015](https://doi.org/10.1152/ajpheart.00178.2015).
- Koch M, Foley JE, Hahn R, Zhou P, Burgeson RE, Gerecke DR, Gordon MK. 2001.** alpha 1(Xx) collagen, a new member of the collagen subfamily, fibril-associated collagens with interrupted triple helices. *Journal of Biological Chemistry* **276(25)**:23120–23126 DOI [10.1074/jbc.M009912200](https://doi.org/10.1074/jbc.M009912200).
- Krieg T, Aumailley M. 2011.** The extracellular matrix of the dermis: flexible structures with dynamic functions. *Experimental Dermatology* **20(8)**:689–695 DOI [10.1111/j.1600-0625.2011.01313.x](https://doi.org/10.1111/j.1600-0625.2011.01313.x).
- Krishnan G, Paul V, Biswas TK, Chouhan VS, Das PJ, Sejian V. 2018.** Adaptation strategies of yak to seasonally driven environmental temperatures in its natural habitat. *International Journal of Biometeorology* **62(8)**:1497–1506 DOI [10.1007/s00484-018-1549-8](https://doi.org/10.1007/s00484-018-1549-8).

- Kuivaniemi H, Tromp G. 2019.** Type III collagen (COL3A1): gene and protein structure, tissue distribution, and associated diseases. *Gene* **707(Pt 3)**:151–171 DOI [10.1016/j.gene.2019.05.003](https://doi.org/10.1016/j.gene.2019.05.003).
- Kular JK, Basu S, Sharma RI. 2014.** The extracellular matrix: structure, composition, age-related differences, tools for analysis and applications for tissue engineering. *Journal of Tissue Engineering and Regenerative Medicine* **5**:2041731414557112 DOI [10.1177/2041731414557112](https://doi.org/10.1177/2041731414557112).
- Kumar VA, Caves JM, Haller CA, Dai E, Liu L, Grainger S, Chaikof EL. 2013.** Acellular vascular grafts generated from collagen and elastin analogs. *Acta Biomaterialia* **9(9)**:8067–8074 DOI [10.1016/j.actbio.2013.05.024](https://doi.org/10.1016/j.actbio.2013.05.024).
- Li J, Meng X, Wang L, Yu Y, Yu H, Wei Q. 2021.** Changes in the expression levels of elastic fibres in yak lungs at different growth stages. *BMC Developmental Biology* **21**:9 DOI [10.1186/s12861-021-00240-w](https://doi.org/10.1186/s12861-021-00240-w).
- Mao M, Alavi MV, Labelle-Dumais C, Gould DB. 2015.** Type IV collagens and basement membrane diseases: cell biology and pathogenic mechanisms. *Current Topics in Membranes* **76(2)**:61–116 DOI [10.1016/bs.ctm.2015.09.002](https://doi.org/10.1016/bs.ctm.2015.09.002).
- Mascaretti RS, Mataloun MM, Dolhnikoff M, Rebello CM. 2009.** Lung morphometry, collagen and elastin content: changes after hyperoxic exposure in preterm rabbits. *Clinics* **64(11)**:1099–1104 DOI [10.1590/S1807-59322009001100010](https://doi.org/10.1590/S1807-59322009001100010).
- Minor AJ, Coulombe KKL. 2020.** Engineering a collagen matrix for cell-instructive regenerative angiogenesis. *Journal of Biomedical Materials Research Part B-applied Biomaterials* **108(6)**:2407–2416 DOI [10.1002/jbm.b.34573](https://doi.org/10.1002/jbm.b.34573).
- Mishra YG, Manavathi B. 2021.** Focal adhesion dynamics in cellular function and disease. *Cell Signaling Technology* **85(5)**:110046 DOI [10.1016/j.cellsig.2021.110046](https://doi.org/10.1016/j.cellsig.2021.110046).
- Mi Y, Wang W, Lu J, Zhang C, Wang Y, Ying H, Sun K. 2018.** Proteasome-mediated degradation of collagen III by cortisol in amnion fibroblasts. *Journal of Molecular Endocrinology* **60(2)**:45–54 DOI [10.1530/JME-17-0215](https://doi.org/10.1530/JME-17-0215).
- Mizuno T, Mickle DA, Kiani CG, Li RK. 2005.** Overexpression of elastin fragments in infarcted myocardium attenuates scar expansion and heart dysfunction. *American Journal of Physiology-Heart and Circulatory Physiology* **288(6)**:H2819–H2827 DOI [10.1152/ajpheart.00862.2004](https://doi.org/10.1152/ajpheart.00862.2004).
- Niu WX, Bao YY, Zhang N, Lu ZN, Ge MX, Li YM, Li Y, Chen MH, He HW. 2022.** Dehydromevalonolactone ameliorates liver fibrosis and inflammation by repressing activation of NLRP3 inflammasome. *Bioorganic Chemistry* **127(7)**:105971 DOI [10.1016/j.bioorg.2022.105971](https://doi.org/10.1016/j.bioorg.2022.105971).
- Pöschl E, Schlötzer-Schrehardt U, Brachvogel B, Saito K, Ninomiya Y, Mayer U. 2004.** Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. *Development* **131(7)**:1619–1628 DOI [10.1242/dev.01037](https://doi.org/10.1242/dev.01037).
- Quesnel C, Nardelli L, Piednoir P, Leçon V, Marchal-Somme J, Lasocki S, Bouadma L, Philip I, Soler P, Crestani B, Dehoux M. 2010.** Alveolar fibroblasts in acute lung injury: biological behaviour and clinical relevance. *European Respiratory Journal* **35(6)**:1312–1321 DOI [10.1183/09031936.00074709](https://doi.org/10.1183/09031936.00074709).
- Rabkin SW. 2017.** The role matrix metalloproteinases in the production of Aortic aneurysm. *Progress in Molecular Biology and Translational Science* **147(10)**:239–265 DOI [10.1016/bs.pmbts.2017.02.002](https://doi.org/10.1016/bs.pmbts.2017.02.002).
- Ricard-Blum S. 2011.** The collagen family. *Cold Spring Harbor Perspectives in Biology* **3(1)**:a004978 DOI [10.1101/cshperspect.a004978](https://doi.org/10.1101/cshperspect.a004978).

- Saito M, Takenouchi Y, Kunisaki N, Kimura S. 2001. Complete primary structure of rainbow trout type I collagen consisting of alpha1(I)alpha2(I)alpha3(I) heterotrimers. *European Journal of Biochemistry* 268(10):2817–2827 DOI 10.1046/j.1432-1327.2001.02160.x.
- Sapkota S, Acharya KP, Laven R, Acharya N. 2022. Possible consequences of climate change on survival, productivity and reproductive performance, and welfare of Himalayan Yak (*Bos grunniens*). *Frontiers in Veterinary Science* 9(8):449 DOI 10.3390/vetsci9080449.
- Schoene RB. 1999. Lung disease at high altitude. *Advances in Experimental Medicine and Biology* 474:47–56 DOI 10.1007/978-1-4615-4711-2.
- Schreier D, Hacker T, Song G, Chesler N. 2013. The role of collagen synthesis in ventricular and vascular adaptation to hypoxic pulmonary hypertension. *Journal of Biomechanical Engineering* 135(2):021018 DOI 10.1115/1.4023480.
- Shi L, Herrmann J, Bou Jawde S, Bates JHT, Nia HT, Suki B. 2022. Modeling the influence of gravity and the mechanical properties of elastin and collagen fibers on alveolar and lung pressure-volume curves. *Scientific Reports* 12:12280 DOI 10.1038/s41598-022-16650-0.
- Sydykov A, Mamazhakypov A, Maripov A, Kosanovic D, Weissmann N, Ghofrani HA, Sarybaev AS, Schermuly RT. 2021. Pulmonary hypertension in acute and chronic high altitude maladaptation disorders. *International Journal of Environmental Research and Public Health* 18(4):1692 DOI 10.3390/ijerph18041692.
- Thenappan T, Prins KW, Pritzker MR, Scandurra J, Volmers K, Weir EK. 2016. The critical role of pulmonary arterial compliance in pulmonary hypertension. *Annals of the American Thoracic Society* 13(2):276–284 DOI 10.1513/AnnalsATS.201509-599FR.
- Voiles L, Lewis DE, Han L, Lupov IP, Lin TL, Robertson MJ, Petrache I, Chang HC. 2014. Overexpression of type VI collagen in neoplastic lung tissues. *Oncology Reports* 32(5):1897–1904 DOI 10.3892/or.2014.3438.
- Volonghi I, Pezzini A, Del Zotto E, Giossi A, Costa P, Ferrari D, Padovani A. 2010. Role of COL4A1 in basement-membrane integrity and cerebral small-vessel disease. The COL4A1 stroke syndrome. *Current Medicinal Chemistry* 17(13):1317–1324 DOI 10.2174/092986710790936293.
- Wan R, Zhao Z, Zhao M, Hu K, Zhai J, Yu H, Wei Q. 2021. Characteristics of pulmonary microvascular structure in postnatal yaks. *Scientific Reports* 11:18265 DOI 10.1038/s41598-021-97760-z.
- Warburton D. 2021. Conserved mechanisms in the formation of the airways and alveoli of the lung. *Frontiers in Cell and Developmental Biology* 9:662059 DOI 10.3389/fcell.2021.662059.
- West CM, Ivy CM, Husnudinov R, Scott GR. 2021. Evolution and developmental plasticity of lung structure in high-altitude deer mice. *Journal of Comparative Physiology B-Biochemical Systems and Environmental Physio* 191(2):385–396 DOI 10.1007/s00360-021-01343-3.
- Wiener P, Robert C, Ahbara A, Salavati M, Abebe A, Kebede A, Wragg D, Friedrich J, Vasoya D, Hume DA, Djikeng A, Watson M, Prendergast JGD, Hanotte O, Mwacharo JM, Clark EL. 2021. Whole-genome sequence data suggest environmental adaptation of ethiopian sheep populations. *Genome Biology and Evolution* 13(3):evab014 DOI 10.1093/gbe/evab014.
- Xin JW, Chai ZX, Zhang CF, Zhang Q, Zhu Y, Cao HW, Ji QM, Zhong JC. 2019. Transcriptome profiles revealed the mechanisms underlying the adaptation of yak to high-altitude environments. *Scientific Reports* 9:7558 DOI 10.1038/s41598-019-43773-8.
- Yamaguchi N, Knaut H. 2022. Focal adhesion-mediated cell anchoring and migration: from in vitro to in vivo. *Development* 149(10):dev200647 DOI 10.1242/dev.200647.
- Yombo DJK, Madala SK, Vemulapalli CP, Ediga HH, Hardie WD. 2023. Pulmonary fibroelastosis—a review. *Matrix Biology* 124(1):1–7 DOI 10.1016/j.matbio.2023.10.003.

- Yurchenco PD. 2011.** Basement membranes: cell scaffoldings and signaling platforms. *Cold Spring Harbor Perspectives in Biology* **3(2)**:a004911 DOI [10.1101/cshperspect.a004911](https://doi.org/10.1101/cshperspect.a004911).
- Zhang WJ, Lin QX, Zhang Y, Liu CT, Qiu LY, Wang HB, Wang YM, Duan CM, Liu ZQ, Zhou J, Wang CY. 2011.** The reconstruction of lung alveolus-like structure in collagen-matrigel/microcapsules scaffolds in vitro. *Journal of Cellular and Molecular Medicine* **15(9)**:1878–1886 DOI [10.1111/j.1582-4934.2010.01189.x](https://doi.org/10.1111/j.1582-4934.2010.01189.x).
- Zhang Y, Zhou M, Liang Y, Li R, Zhang L, Chen S, Yang K, Ding H, Tan X, Zhang Q, Qiao Z. 2023.** Study of transcriptomic analysis of yak (*Bos grunniens*) and Cattle (*Bos taurus*) pulmonary artery smooth muscle cells under oxygen concentration gradients and differences in their lung histology and expression of pyruvate dehydrogenase Kinase 1-related factors. *Animals* **13(22)**:3450 DOI [10.3390/ani13223450](https://doi.org/10.3390/ani13223450).
- Zhao P, Zhao F, Hu J, Wang J, Liu X, Zhao Z, Xi Q, Sun H, Li S, Luo Y. 2022.** Physiology and transcriptomics analysis reveal the contribution of lungs on high-altitude hypoxia adaptation in Tibetan sheep. *Frontiers in Physiology* **13**:885444 DOI [10.3389/fphys.2022.885444](https://doi.org/10.3389/fphys.2022.885444).
- Zhou JX, Yu SJ, He JF, Cui Y. 2015.** Comparative analysis of the structural organization and morphology of pulmonary arteries in the lungs of adult yaks and cattle. *Chinese Journal of Veterinary Medicine* **35(11)**:1840–1844 1862 DOI [10.16303/j.cnki.1005-4545.2015.11.22](https://doi.org/10.16303/j.cnki.1005-4545.2015.11.22).
- Zulliger MA, Fridez P, Hayashi K, Stergiopoulos N. 2004.** A strain energy function for arteries accounting for wall composition and structure. *Journal of Biomechanics* **37(7)**:989–1000 DOI [10.1016/j.jbiomech.2003.11.026](https://doi.org/10.1016/j.jbiomech.2003.11.026).