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Research paper

Transcriptomic analysis provides insight into high-altitude acclimation in domestic goats

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ABSTRACT

Domestic goats are distributed in a wide range of habitats and have acclimated to their local environmental conditions. To investigate the gene expression changes of goats that are induced by high altitude stress, we performed RNA-seq on 27 samples from the three hypoxia-sensitive tissues (heart, lung, and skeletal muscle) in three indigenous populations from distinct altitudes (600 m, 2000 m, and 3000 m). We generated 129 Gb of high-quality sequencing data (~4 Gb per sample) and catalogued the expression profiles of 12,421 annotated hircine genes in each sample. The analysis showed global similarities and differences of high-altitude transcriptomes among populations and tissues as well as revealed that the heart underwent the most high-altitude induced expression changes. We identified numerous differentially expressed genes that exhibited distinct expression patterns, and nonsynonymous single nucleotide variant-containing genes that were highly differentiated between the high- and low-altitude populations. These genes have known or potential roles in hypoxia response and were enriched in functional gene categories potentially responsible for high-altitude stress. Therefore, they are appealing candidates for further investigation of the gene expression and associated regulatory mechanisms related to high-altitude acclimation.

1. Introduction

To date, over 1000 domestic goat breeds (*Capra hircus*) are widely reared worldwide, especially in Asia (~500 million (M) heads), Africa (~290 M heads), Europe (~21 M heads), and North America (~3 M heads) (Aziz, 2010). The agricultural, economic, cultural, and biomedical importance of domestic goats has led to significant efforts to decode the goat genome and its genetic components (Amills, 2014). Sequencing and analysis of the Chinese domestic Yunnan black goat by the International Goat Genome Consortium provided a valuable resource that could expedite practical applications of gene mapping and marker-assisted breeding in goats, and improve the utility of the goat as a biomedical model and bioreactor (Dong et al., 2013).

Approximately 62 indigenous goat breeds in China are distributed in a wide range of habitats, from the extremely inhospitable Tibetan Plateau to the warm and humid regions of southwest China. This has led to remarkable phenotypic diversity (Ajmone-Marsan et al., 2014; Liu et al., 2009; Zeder and Hesse, 2000), making these breeds valuable resources for comparatively investigating the transcriptomic variations induced by varying local environmental conditions. The harsh high-altitude environment (typically, hypoxia, low temperature, high solar radiation, and lack of biological production) imposes strong selective pressures on high altitude species. Candidate adaptive genes for high altitude are comprehensively documented in humans (Bigham et al., 2009; Huerta-Sánchez et al., 2013; Simonson et al., 2010; Yi et al., 2010), yaks (Qiu et al., 2012), ground tits (Qu et al., 2013), Tibetan antelopes (Ge et al., 2013), snow leopards (Cho et al., 2013), Tibetan wild boars (Li et al., 2013), Tibetan mastiffs (Gou et al., 2014), and snub-nosed monkeys (Zhou et al., 2014). This has greatly enhanced our understanding of the complex genetic architecture that underlies high-altitude adaptation. Among different geographical populations of highland residents (Andean in South America (Bigham et al., 2009), Tibetan in Asia (Simonson et al., 2010; Yi et al., 2010), and Ethiopian in Africa (Huerta-Sánchez et al., 2013)),

Abbreviations: SNV, single nucleotide variant; DEG, differentially expressed gene; GO, gene ontology; CC, cellular component; MF, molecular function; BP, biological process; Q-PCR, quantitative polymerase chain reaction.

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although the master biological pathway of the cellular response to hypoxia implicated in each continental region is consistent (hypoxia-inducible factor 1, *HIF1*), the causal loci are often different. This illustrates that specific convergent adaptive scenarios are triggered by strong selective pressures in high-altitude harsh environments (Scheinfeldt and Tishkoff, 2013). Nonetheless, information concerning gene expression change response to high-altitude stress in goat is long overdue.

To explore the exceptional mechanisms of gene expression that are induced by high altitude environments in goats, we performed a comparative transcriptomic analysis of three hypoxia-sensitive tissues (heart, lung, and skeletal muscle) in three indigenous populations that reside in geographically neighboring regions with distinct altitudes (600 m, 2000 m, and 3000 m) (Fig. 1a). We identified numerous differentially expressed genes and non-synonymous single nucleotide variant (SNV)-containing genes that are over-represented in hypoxia-related functional gene categories and are likely the consequence of hypoxia-acclimation in these goats.

2. Materials and methods

2.1. Animals and sample collection

Three adult females (~3 years old) for each of the three indigenous goat populations with distinct altitudes in southwest China (600 m, 2000 m, and 3000 m) were used in this study (Fig. 1a). Animals were humanely killed to ameliorate suffering. All of the animals and samples used in this study were collected according to the guidelines for the care and use of experimental animals established by the Ministry of Agriculture of China.

Three representatively hypoxia-sensitive tissues (i.e., heart, lung and skeletal muscle) were rapidly separated from each carcass, immediately frozen in liquid nitrogen, and stored at −80 °C until RNA extraction.

2.2. RNA isolation, library preparation, and sequencing

Total RNA was extracted using Trizol reagent (Life Technologies, Beijing, China) according to the manufacturer's protocols. Sequencing

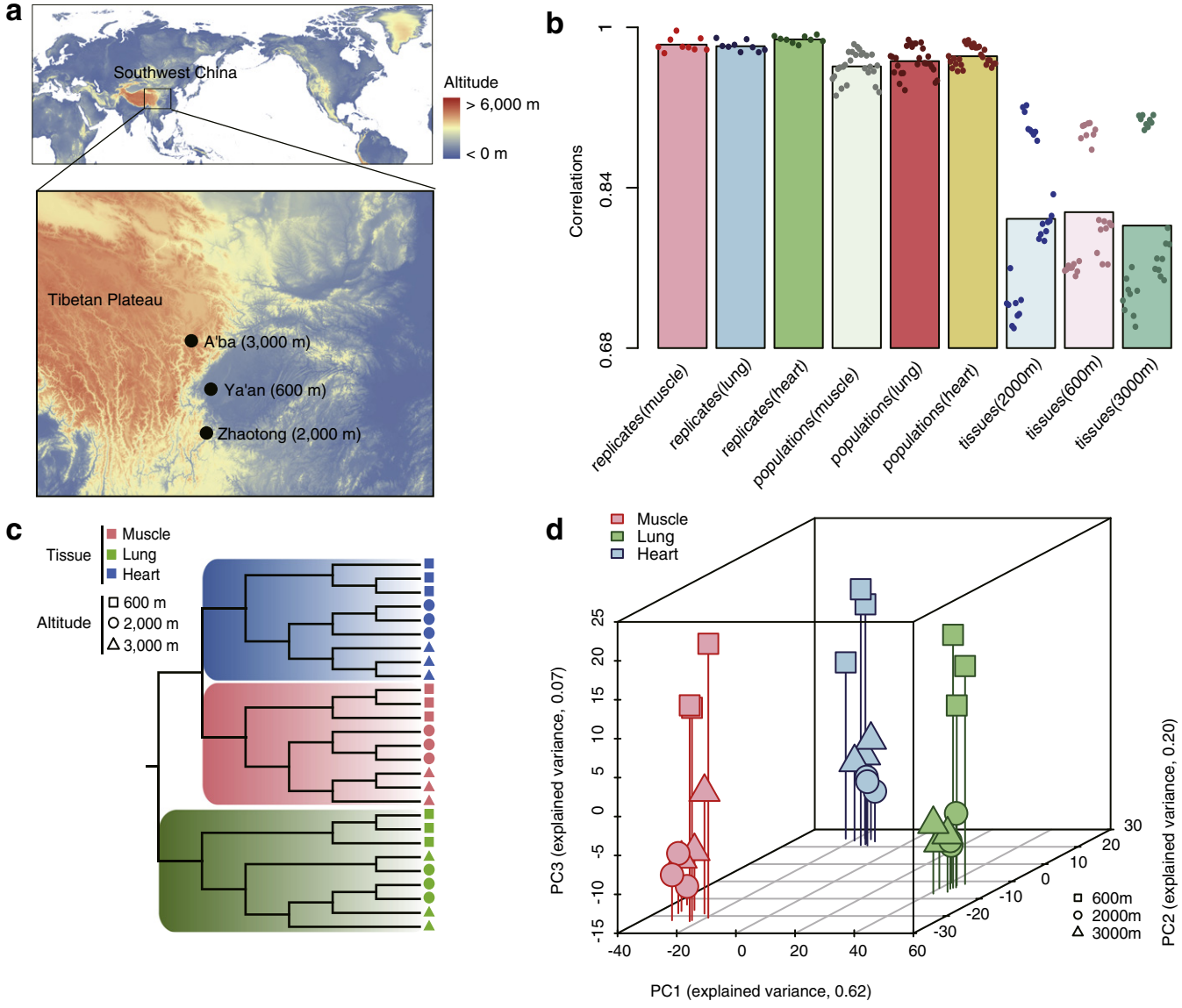

Fig. 1. Characteristics of high-altitude transcriptomes in goats. (a) Geographic locations of the studied indigenous domestic goats. (b) Comparison of pairwise Pearson's correlation for gene expression profiles between samples. The correlation between every two samples was calculated by $\log_2$-transformed FPKM gene expression values. Then, these correlation rates were grouped into the following categories: biological replicates ($n = 9$), the same populations ($n = 27$), and the same tissues ($n = 27$). (c) Hierarchical clustering of samples. (d) Three-way PCA plot of samples. The fraction of the variance explained is 61.95%, 19.57%, and 6.88% for eigenvectors 1, 2, and 3, respectively.