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Comparative proteomic analyses using iTRAQ-labeling provides insights into fiber diversity in sheep and goats

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ABSTRACT

The structural component of wool and hair fibers, such as keratin-associated proteins (KAPs), has been well described, but the genetic determinants of fiber diameter are largely unknown. Here, we have used an iTRAQ-based proteomic approach to investigate differences in protein abundance among 18 samples from sheep and goats across a diverse range of fibers. We identified proteins with different abundance and are associated with variation in fiber features. Proteins with different abundance are mainly keratin or keratin-associated proteins (KRTAP11-1, KRT6A, KRT38), or are related to hair growth (DSC2, DSG3, EEF2, CALML5, TCHH, SELENBP1) and fatty acid synthesis (FABP4, FABP5). RNA-seq further confirmed the functional importance of the *DSC2* gene in the determination of woolly phenotype in goat fibers. This comprehensive analysis of fibers from major fiber-producing animals is the first to provide a list of candidate proteins that are involved in fiber formation. This list will be valuable asset for future studies into the molecular mechanisms that underlie fiber diversity.

Biological significance: Proteins are the basis for animal-derived hair fibers, yet proteins conferring fiber structure and characteristics in sheep and goats are largely elusive. By examining 27 fibers samples representing 9 fiber types from sheep and goats through the iTRAQ approach, we show a list of differentially abundant proteins that are important to hair structural component, or genes related to hair growth and fatty acid synthesis. RNA-seq further validated the *DSC2* gene is key to the woolly/straight hair phenotype in goats.

1. Introduction

The use of keratinous protein fibers for clothing and other textile products has long accompanied human life [1], with the vast majority of animal fibers coming from domestic sheep and goats [2]. The quality and value of textiles largely depends on the color, diameter and length, origin and availability of the fibers [3]. For example, the price of fine cashmere from goats can be 18–30-fold higher than that of wool from Merino sheep [3]. Fiber diameter is one of the most important traits related to quality and it varies enormously among species, and even within a single breed. However, the genetic determinants of fiber diversity (*e.g.* diameter, curvature) in sheep and goats have not been characterized.

Proteins are the basis for animal-derived filaments and hair fibers. Mammalian hair fibers are complex, including a large amount of keratin and keratin associated proteins (KAPs) [4], as well as lipids and carbohydrates [5]. Previous studies have uncovered a number of proteins that contribute to wool composition and structure [6,7], and that can also be found in dissected hair follicles [8]. For example, 113 proteins were identified in wool fibers [6], and 108 proteins were identified in the wool cuticle [7]. Most of the proteins identified were in the keratin family, a key family in our understanding the fiber structure and characteristics. However, these initial studies were limited to protein extraction methodology [9], and 2D gel electrophoresis analysis [6;7;8], so were not able to reveal lower-abundance proteins. A new quantitative proteomic approach, the isobaric tag for relative and absolute quantitation (iTRAQ), is capable of simultaneously identifying and quantifying proteins from multiple samples, while retaining important post-translational modification (PTM) information, and remain unbiased toward proteins not amenable to traditional proteomic studies that rely on two-dimensional electrophoresis (2-DE) techniques [10,11]. For instance, quantitative comparison using iTRAQ has revealed an interesting correlation between dietary restriction and wool protein composition and quality [12].

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This inspired us to explore differences in protein constitution among fiber samples with extreme phenotypes from domesticated sheep and goats, and the iTRAQ method was used to systematically characterize the proteome profiles using varied fiber samples. In this study, we identified a list of fiber-specific proteins that are candidates for explaining fiber diversity, of which many are previously reported proteins related to keratin, hair follicle (HF) development, and fatty acid metabolism. We further validated functional genes underlying fiber differences in goats through RNA-seq. Our results provide new insights into the molecular mechanisms of fiber formation in fiber-producing animals.

2. Materials and methods

2.1. Fiber samples

All animal protocols were approved by the College of Animal Sciences of Northwest A & F University. The hair sampling procedures in the present study had received prior approval from the Experimental Animal Manage Committee of Northwest A & F University (Approval ID: 2014YB008-01). To ensure a range of fiber diameters, we collected wool from Chinese Merino and Small-tail Han sheep, as well as mohair from Angora goats and cashmere goats (Fig. 1A–H). Specifically, the Small-tail Han, a Mongolian genotype, was developed in the semi-humid agricultural areas of China and features high prolificity. It also produces two types of fibers (coarse wool and wool) that are mainly used for carpet. Cashmere goat skin has two distinct types of hair follicle, the primary hair follicle (PHF) which produces guard hair, and the secondary hair follicle (SHF) which produces cashmere. The diameter of these six hair fibers varies largely (Fig. 1I).

We collected samples from three individuals in each breed. Importantly, the wool samples were obtained from healthy ewes (~2 years old) in May 2015, at shearing. Fiber samples collected from the side of the rear of body and closest to the skin were chosen for further analysis.

2.2. Protein extraction, quantification and digestion

Proteins were extracted from the samples using the method described [13]. Briefly, fiber samples were scoured with 0.15% Teric GN9 at 60 °C to remove dust and residual grease; they were rinsed in double distilled H_2O and cut into small pieces and ground into a powder in liquid nitrogen. A 20 mg sub-sample of the powder was extracted in 500 µL lysis buffer (7 M urea, 2 M thiourea, 50 mM Tris, 50 mM TCEP; pH 4) at 37 °C overnight with uninterrupted shaking. Insoluble particles were then separated by centrifugation at 13,000*g* for 15 min at room temperature, and the soluble fraction was concentrated and purified by the methanol/chloroform method [14]. The entire pellets precipitated from the soluble fraction were then digested with trypsin. They were separately reduced in 50 µL of 50 mM TCEP in 100 mM ammonium bicarbonate (pH 8) at 56 °C for 45 min, followed by alkylation in 50 µL of 360 mM acrylamide in 100 mM ammonium bicarbonate for 30 min. Trypsin (1 µg in 100 µL of 50 mM ammonium bicarbonate) was added and the extract digested at 37 °C for 18 h. Protein quality and concentrations were determined with SDS-PAGE (Fig. S1), and the 2-D Quant Kit (General Electric Company, Fairfield, CT, USA) following the manufacturer's instructions.

2.3. iTRAQ labeling, SCX fractionation, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

For iTRAQ analysis, protein was digested with Trypsin Gold (Promega, Madison, WI, USA): 4 µL trypsin (0.5 µg/µL) was added to 120 µg of protein solution for digestion at 37 °C for 16 h, and the resulting peptides were dried in a centrifugal vacuum concentrator. Proteins from 18 fiber samples were labeled with iTRAQ tags, with groups of three samples clustered for labeling following the manufacturer's protocol for the iTRAQ 8-plex labeling kit (Applied Biosystems, Foster City, CA, USA). Detailed labeling information is summarized in Supplementary Table S1.

Labeled samples were allocated into two subgroups, mixed and subjected to strong cationic-exchange (SCX) chromatography on a HPLC Pump system (LC-20AB, Shimadzu, Kyoto, Japan) equipped with

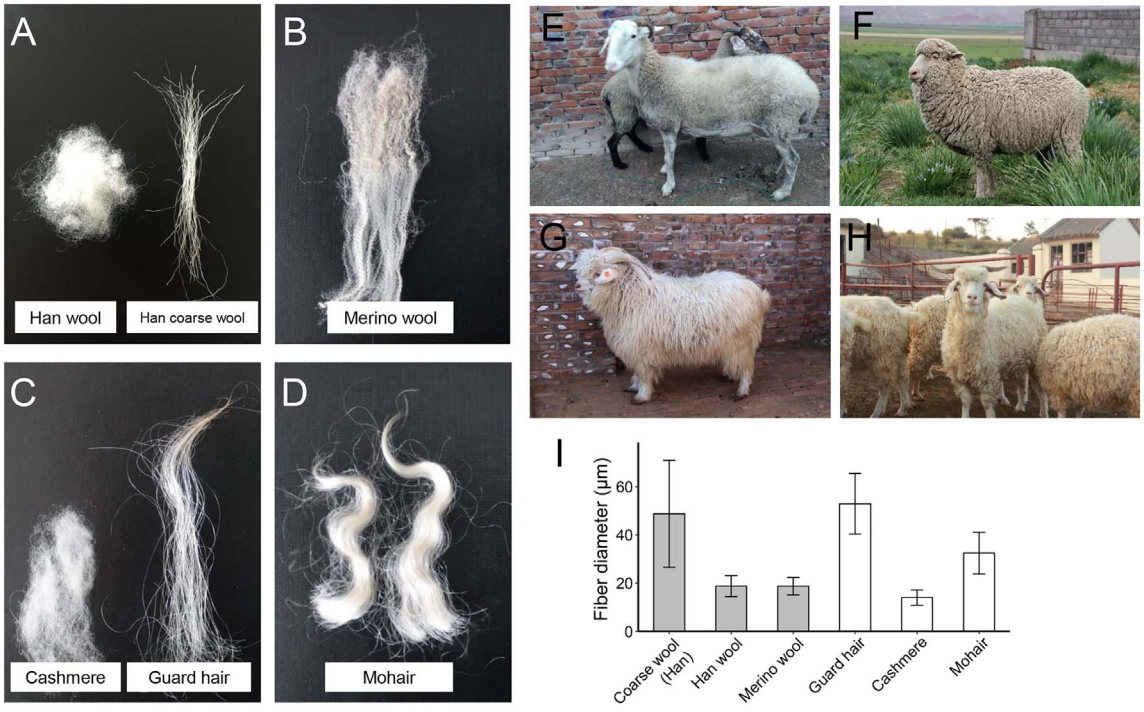

Fig. 1. The appearance of fibers from Small-tail Han sheep (A), Chinese Merino sheep (B), Shaanbei Cashmere goats (C), and Angora goats (D). Animal breeds used for sampling, Small-tail Han sheep (E), Chinese Merino (F), Shaanbei Cashmere (G), Angora (H). (I) The diameter of hair fibers used in this study.