ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

doi: 10.1111/age.12701

Transcriptomic analysis reveals differentially expressed genes associated with wool length in rabbit

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Summary

Rabbit fur characteristics are primarily genetically determined traits. We used Illumina high-throughput sequencing technology to assess gene expression in the skin tissues of rabbits derived from a cross between Wanxi Angora rabbits and Rex rabbits, which exhibit differential characteristics of short and long wool respectively, to investigate molecular mechanisms related to wool length determination. To identify key regulatory genes involved in rabbit wool length, genes that were differentially expressed between the long- and short-wool rabbits based on a P-value < 0.05 and $\log_2|\text{fold change}| > 1$ were characterized. A total of 798 genes were up-regulated and 523 were down-regulated in the long-wool group compared to expression levels in the short-wool group, and these genes were annotated with GO terms and KEGG pathways, revealing wool-development-related biological functions. The Wnt, Hedgehog and TGF-β signaling pathways, which are related to cell proliferation, fibroblast proliferation and hair follicle regulation respectively, were identified. The expression levels of eight genes were validated by RT-qPCR. In addition, an interaction network was constructed to show the regulatory relationships among the differentially expressed genes. In this study, we found that *FGF5*, *WNT5A*, *BMP4* and *BMP7* showed significant differential expression between the two groups. These transcriptomic profiling results provide comprehensive gene expression information for improving understanding of the molecular mechanisms involved in the growth and development of rabbit wool.

Keywords candidate gene, hair follicle development, transcriptome, *Oryctolagus cuniculus*

Introduction

Wool length is an important trait in rabbit production. Rabbit wool quality is affected by many factors such as breed, management, climate and physiology. At present, most studies of hair growth have focused on humans, mice and sheep (He *et al.* 2016; Smith *et al.* 2016). However, there are a limited number of studies regarding the mechanisms responsible for wool development in rabbits. Transcriptomics is a powerful tool for the identification of functional genes in different tissues and states (Kim *et al.* 2006); hence, studies based on Illumina high-throughput sequencing technology could allow for the further exploration of the biological mechanisms of hair growth.

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Accepted for publication 28 May 2018

Wool is formed by keratinocytes, which are generated from a progenitor population at the bottom of the hair follicle (Chi *et al.* 2013). Dermal papilla (DP) cells are populations of mesenchymal cells at the base of HFs (Driskell *et al.* 2011) that provide signals that help specify the shape, size and pigmentation of wool (Enshell-Seijffers *et al.* 2010). Wool growth is regulated by several cytokines including insulin-like growth factor-1 (IGF1), epidermal growth factor (EGF), fibroblast growth factor (FGF), keratin, keratin-associated protein (KAP) and transforming growth factor (TGF), among others (Murillas *et al.* 1995; Rosenquist & Martin 1996; Oshimori & Fuchs 2012; Gong *et al.* 2016; Darwish *et al.* 2017).

In cashmere goats, DP cells have been isolated from primary hair follicles and secondary hair follicles (Zhu *et al.* 2014). FGF5 and its receptor, FGFR1, are expressed in both types of DP cells (Ota *et al.* 2002). In addition, adenovirus-mediated overexpression of FGF5 may up-regulate the mRNA expression of *IGF1*, but the expression of anagen chalone bone morphogenetic protein 4 (BMP4) is down-regulated in DP cells (He *et al.* 2016). In the regulation of the anagen–catagen transition, FGF5s acts as an inhibitor

of FGF5 in cashmere goat DP cells (He *et al.* 2016). Most of these genes are related to the Wnt signaling pathway, which is necessary for HF development and stimulates the growth (anagen) phase of the hair cycle. Activation of the Wnt pathway would thus cause further stimulation of anagen signaling and hair growth. Most of the strategies that have been used to induce constitutive Wnt activation to enhance hair growth have led to neoplastic transformation of the epithelial hair matrix (Smith *et al.* 2016).

In this study, Illumina high-throughput RNA sequencing of Wanxi Angora and Rex rabbits was used to identify differentially expressed genes (DEGs) involved in fur-development-related pathways. Our results provide a better understanding of the molecular mechanisms of wool length in rabbits.

Materials and methods

Sample collection

Rabbits were acquired from the Anhui Academy of Agricultural Sciences and were reared in a controlled environment. Full-sibling families from a cross between Wanxi Angora rabbits (long wool) and White Rex rabbits (short wool) were obtained, and significant separation was observed in terms of wool length, with all the offspring having a white coat color, three rabbits having long (L) wool and the rest having short (S) wool. Rabbits of the two phenotypes were housed under the same conditions, including identical forage and temperature conditions. All rabbits used for testing were fed with pellet feed and grass. Healthy L- and S-group rabbits were collected ($n = 3$ per group) at eight months of age, and the back wool length of each group was measured (Fig. 1). Furthermore, Wanxi Angora rabbits, White Rex rabbits and Chinchilla Rex rabbits were reared under the same conditions for evaluating the expression levels of genes associated with wool length.

For anesthesia, an ear vein injection of 0.7% pentobarbital sodium (6 ml/kg) was administered, and respiration was not inhibited by muscle relaxants in the anesthetic. Under anesthesia, skin tissue (1 cm^2) was collected from the back of each rabbit, placed immediately in liquid nitrogen and stored at −70 °C until use. To prevent bacterial infection, iodine solution was placed on the wound. The experimental procedures in this study were approved by the Animal Care and Use Committee at Yangzhou University.

cDNA library preparation, Illumina sequencing and transcriptome mapping

Total RNA was extracted from each sample using a mirVana™ miRNA Isolation Kit (#AM1561, Ambion), following the manufacturer's instructions. An Agilent Bioanalyzer 2100 (Agilent Technologies) was used to ensure RNA integrity by determining the RNA integrity number. Eligible total RNA was further purified by an RNeasy micro kit (Cat#74004, QIAGEN, GmBH) and RNase-Free DNase Set (Cat#79254, QIAGEN, GmBH). Then, cDNA libraries for the L and S groups were constructed, and a Qubit® 2.0 fluorometer and Qubi dsDNA HS kit were used to determine library concentration. Finally, clusters were generated, and the sequencing reagent was prepared according to the HiSeq 2500 user guide using paired-end technology. We analyzed the data in real time using data collection software (Illumina, Inc.) for Illumina sequencing.

Raw sequencing data were conducted using FASTX (version 0.0.13). Low-quality reads shorter than 20 nucleotides and 3′ end bases with quality scores ($Q = -10 \times \log_{error\_ratio}$) lower than 10 were removed. TOPHAT (version 2.0.9) (Trapnell *et al.* 2009) was used to map the clean reads to the *Oryctolagus cuniculus* genome from Ensembl OryCun2.0, allowing two mismatches and

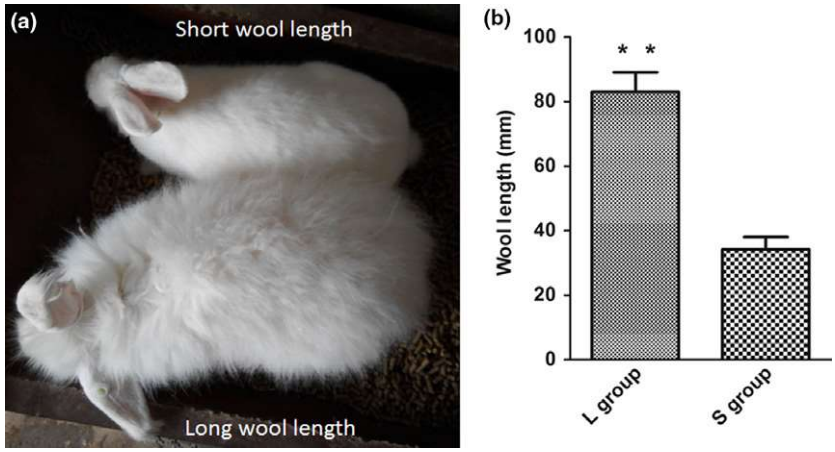

Figure 1 Image and wool length data of long- and short-wool rabbit groups. (a) Phenotypic appearance of long-wool (L) and short-wool (S) rabbits. (b) Bar plot of wool lengths of L and S groups. Error bars indicate the mean ± SD of triplicate experiments. $^{**}P < 0.01$.

multiple hits (≤2). CUFFLINKS (version 2.1.1; Trapnell *et al.* 2010) was used to calculate the expression of transcripts.

Identification of DEGs and functional annotation

To identify gene expression levels that were significantly different between L- and S-group rabbits, the fragments per kilobase of exon per million fragments mapped (FPKM) method was used for estimating gene expression levels (Mortazavi *et al.* 2008). A P-value < 0.05 and $\log_2$|fold change| > 1 were considered statistically significant. BLAST2GO software was used to perform functional annotation according to gene ontology (GO) terms (http://www.geneontology.org), and annotated genes were assigned to the molecular function, cellular component and biological process categories using WEGO (http://wego.genomics.org.cn) (Ye *et al.* 2006). DEGs were mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/) (Kanehisa & Goto 2000) using BLAST, and KEGG pathways were annotated. Enrichment analysis of the main signal transduction pathways and biochemical metabolic pathways was performed. The STRING database was used to perform protein–protein interaction network analysis using the union of all DEGs between the L and S groups to build the network.

Quantitative real-time RT-qPCR validation

Eight known genes associated with wool development were selected from among the DEGs for validation, and four candidate genes were chosen for the assessment of gene expression in rabbit breeds with different wool lengths by real-time RT-qPCR analysis. Each reverse transcription reaction consisted of 2.5 μg of RNA and 2 μl of 5× gDNA buffer, with ddH_2O added to 10 μl. The reaction was incubated at 42 °C for 3 min. Then, 2 μl of 10× Fast RT Buffer, 1 μl of RT Enzyme Mix, 2 μl of qFQ-qRT Primer Mix and 5 μl of ddH_2O were added to the 10-μl mixture followed by incubation at 42 °C for 15 min and 95 °C for 3 min to synthesize cDNA. RT-qPCR was performed in a 7500 Real-Time PCR System (Applied Biosystems) using AceQ qPCR SYBR® Green Master Mix (Vazyme). The 20-μl PCR reaction mixture consisted of 2 μl of cDNA, 10 μl of AceQ™ qPCR SYBR Green Master Mix, 0.4 μl of 50× ROX Reference Dye 2, 0.4 μl of forward primer, 0.4 μl of reverse primer and 6.8 μl of ddH_2O. The specific primer sequences are listed in Table S1. Samples were analyzed in triplicate. At the end of the PCR, melting curves were obtained and analyzed at the third stage to validate the specific generation of the desired PCR products. mRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen 2001), with *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) used as a reference gene.

Results and discussion

Illumina transcriptome sequencing

The six skin samples (three from L-group and three from S-group rabbits) were sequenced using the Illumina sequencing platform. We obtained 40.8–48.3 million raw reads from each library and 38.9–46.0 million high-quality clean reads. The percentage of clean reads was >95% for each sample after trimming (Table 1). All reads were deposited in the Short Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) with the accession number SRR4925734.

TOPHAT spliced mapping (Trapnell *et al.* 2009) is suitable for eukaryotic (including introns) transcriptome sequencing and was used for genome mapping. The percentage of reads mapping to each gene region is shown in Fig. S1, with gene and coding regions accounting for the majority of reads in the samples. To identify genes differentially expressed between the S and L samples, the RPKM method was used to calculate gene expression levels. Significant DEGs were determined based on a P-value < 0.05 and $\log_2$|fold change| > 1 (Robinson *et al.* 2009). A total of 1321 significant DEGs were identified (Table S2), including 523 that were down-regulated and 798 that were up-regulated in the L group compared to levels in the S group. Hierarchical clustering showed that gene expression patterns between the two groups were distinguishable, indicating that gene expression patterns in the L group were different from those in the S group

Table 1 Summary of RNA-seq for each sample.

Sample ID	Raw reads	Quality trimmed	Adaptor trimmed	Clean reads	Clean ratio (%)	rRNA ratio (%)
S1	42 302 698	41 999 005	41 275 815	40 284 926	95.2	0.2
S2	44 444 918	44 100 621	43 325 935	42 244 946	95.1	0.6
S3	40 898 814	40 587 677	39 886 034	38 910 394	95.1	0.5
L1	48 327 788	48 003 368	47 146 246	46 004 332	95.2	0.2
L2	47 849 748	47 537 106	46 683 806	45 555 688	95.2	0.3
L3	44 982 450	44 671 912	43 883 066	42 818 914	95.2	0.1

Clean ratio (%) = (clean reads/raw reads) × 100. S samples belong to the short wool group; L samples belong to the long wool group.

(Fig. 2). The relationship between the fold change and significance of each DEG was examined with volcano plots (Fig. 3).

We performed qRT-PCR for eight DEGs, six that were up-regulated (*WNT5A*, *WNT11*, *BMP4*, *BMP7*, *MSX2* and *FGF5*) and two that were down-regulated (*PDGFA* and

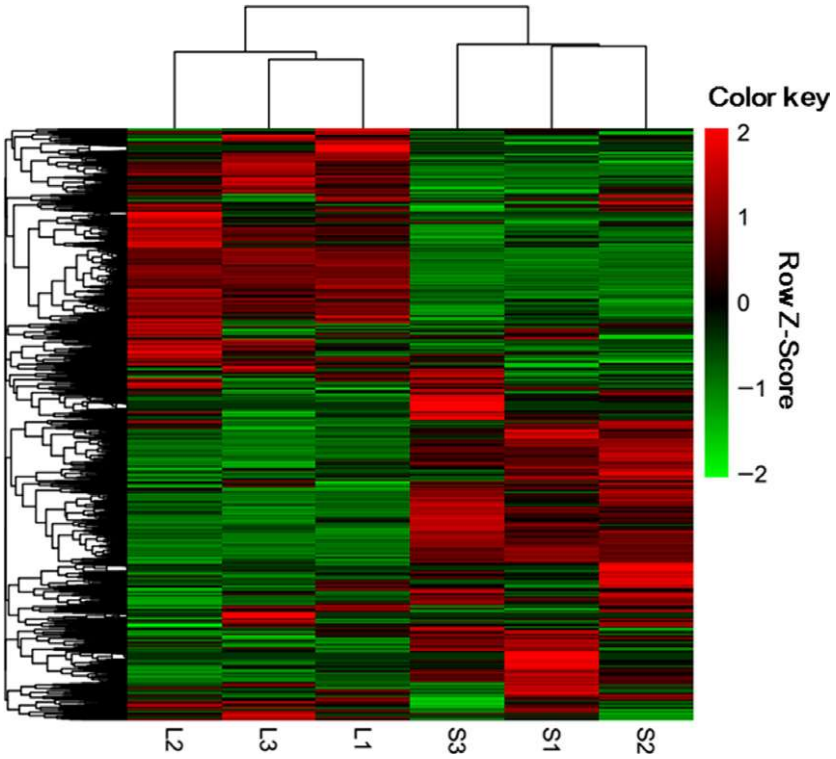

Figure 2 Heatmap showing gene expression profiles. Cluster analysis of gene expression levels in long-wool (L) and short-wool (S) groups.

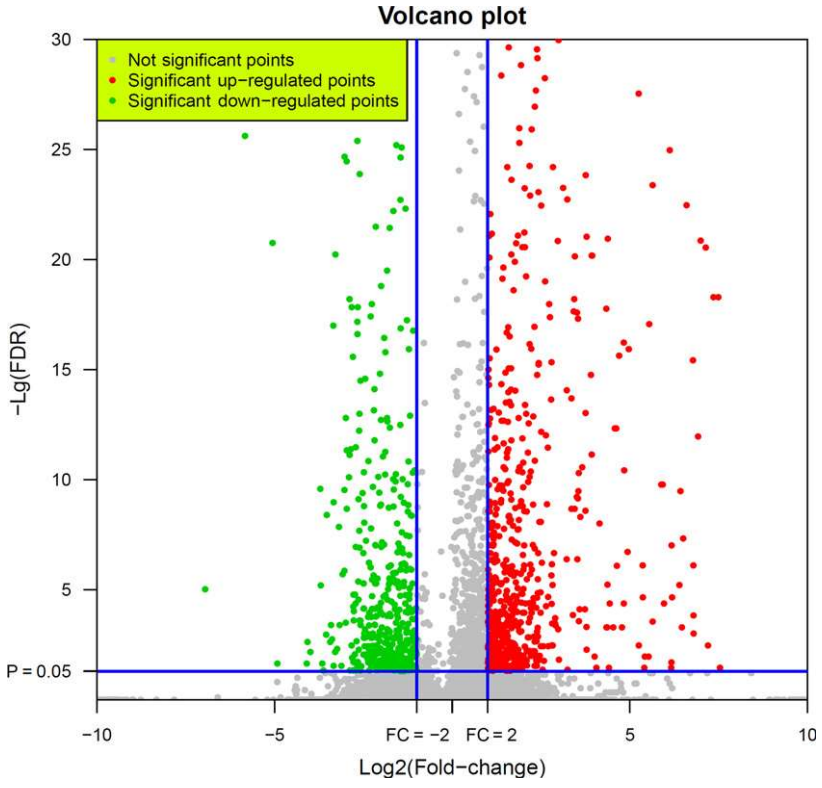

Figure 3 Volcano plot of differentially expressed genes, comparing gene expression levels between the short-wool and long-wool groups. The criteria *P*-value ≤ 0.05 and $\log_2$ |fold change| 1 were used to filter DEGs. Green dots indicate down-regulated genes and red dots represent up-regulated genes; gray dots represent genes that did not show any significant difference between the short-wool and long-wool group samples.

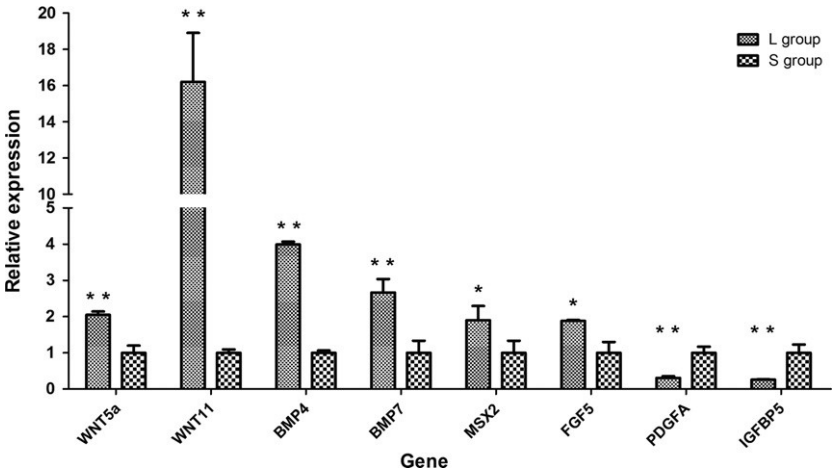

Figure 4 Validation of differentially expressed genes between short-wool and long-wool groups of rabbits. RT-qPCR validation of *WNT5A*, *WNT11*, *BMP4*, *BMP7*, *MSX2*, *FGF5*, *FGF18*, *PDGFA* and *IGFBP5* mRNA levels in skin samples from short-wool and long-wool rabbits. The expression levels of genes in the long-wool group were normalized to those in the short-wool group. Error bars indicate the mean ± SD of triplicate experiments. $*P < 0.05$; $**P < 0.01$.

IGFBP5) in the L group compared to levels in the S group, to validate the Illumina sequencing results. The results of qRT-PCR were consistent with those obtained by Illumina sequencing (Fig. 4).

Functional annotation and pathway analysis

For functional analysis, 1141 genes differentially expressed between the L and S groups were annotated with GO terms (Table S3), and 394 DEGs were identified in 241 KEGG pathways (Table S4). For GO term analysis, DEGs were categorized into 55 functional groups (Fig. S2), and the most represented GO term in each of the three main categories of cellular component, molecular function and biological process were cell and cell part, binding and cellular process respectively. As expected, DEGs annotated with the GO terms positive regulation of cell proliferation (GO:0008284), positive regulation of endothelial cell proliferation (GO:0001938), negative regulation of canonical Wnt signaling pathway (GO:0090090), positive regulation of epithelial cell proliferation (GO:0050679) and positive regulation of fibroblast proliferation (GO:0048146) were found in the biological process category. The results of our transcriptomic analysis thus provide a valuable resource for the investigation of specific functional processes and pathways involved in rabbit wool growth.

In the KEGG pathway analysis, DEGs were categorized into 31 functional groups (Fig. S3), the most represented of which were signal transduction, immune system, and global and overview maps. Interestingly, included in the signal transduction category, we found several wool-development-related pathways, such as the TGF-β, Wnt and Hedgehog signaling pathways. Wnt, bone morphogenic protein (BMP), TGF-β and FGF proteins play important roles in HF-related molecular signaling pathways. TGF-β is known to promote the withdrawal of keratinocytes from the cell cycle (Shi & Massagué 2003). Specifically, TGF-β2 was detected in the transition between the growth and destructive phases of the adult hair cycle, and research initially concentrated on the role of TGF-β family members in the cessation of growth and/or induction of apoptosis (Soma *et al.* 2003).

By comparing the results of GO functional enrichment and KEGG pathway analysis, we identified several genes in common pathways (Wnt and Hedgehog signaling

Table 2 Putative candidate genes based on DEGs associated with wool length.

Gene symbol	Gene ID	Fold change (L/S)	Up/down-regulated in L group
FGF5	ENSOCUG00000012418	15.21244778	Up
MSX2	ENSOCUG00000009161	28.62721785	Up
TGFA	ENSOCUG00000029636	10.26174642	Up
CNPY4	ENSOCUG00000000306	2.853411132	Up
SHH	ENSOCUG00000016862	20.56544702	Up
WNT5A	ENSOCUG00000000380	5.154036332	Up
SFRP1	ENSOCUG00000026917	4.064583712	Up
EIF2B4	ENSOCUG00000017076	2.712002412	Up
BAMBI	ENSOCUG00000007403	57.81951655	Up
BMP4	ENSOCUG00000011097	3.43827236	Up
BMP2K	ENSOCUG00000011578	3.326345535	Up
BMP7	ENSOCUG00000025711	2.172839109	Up
WLS	ENSOCUG00000000532	3.706212393	Up
WNT11	ENSOCUG00000001726	3,378726994	Up
PDGFRL	ENSOCUG00000005211	0.420973649	Down
PDGFRB	ENSOCUG00000001725	0.324363014	Down
PDGFA	ENSOCUG00000005660	0.319368234	Down
IGFBP6	ENSOCUG00000001412	0.496559657	Down
IGFBP5	ENSOCUG00000025513	0.271963675	Down
ITGBL1	ENSOCUG00000008813	0.430671547	Down
VEGF	ENSOCUG00000011610	0.332139253	Down
FGF18	ENSOCUG00000011124	0.077077803	Down
DKK3	ENSOCUG00000004841	0.162835583	Down
GREM1	ENSOCUG00000005596	0.076303982	Down
WNT5B	ENSOCUG00000013686	0.242809123	Down
WNT4	ENSOCUG00000022473	0.217105436	Down
TGFBR2	ENSOCUG00000015963	0.408570167	Down
TGFBR3	ENSOCUG00000016083	0.262333219	Down
TGFBI	ENSOCUG00000004928	0.177855699	Down

L group denotes long-wooled group of rabbits.

pathways) that are related to wool length and HF growth (Table 2, Fig. 5) including *FGF5*, *IGF1*, *BMP4*, *FGF18*, *WNT5A*, *WNT5B* and *WNT11*. Many studies have shown that these genes have effects on HF morphogenesis and the hair cycle in mice and humans, and they may control the development of the DP and matrix (Bostjancic & Glavac 2008; Driskell *et al.* 2011; Lee & Tumbar 2012). Many proteins, such as BMP-2/4/7, Wnt/β-catenin, Ctgf/Ccn2, EGF, Noggin/Lef-1, Dkk1/Dkk4 and sonic hedgehog, have been shown to be involved in signaling between the matrix and the DP (Wang *et al.* 2012; Chi *et al.* 2013). The Wnt signaling pathway is involved in the biological process of skin growth and development, particularly Wnt/β-catenin signaling. Wnt family genes, such as *WNT5A* and *WNT11*, were highly expressed in the L group. These Wnt family genes could be activators promoting epidermis

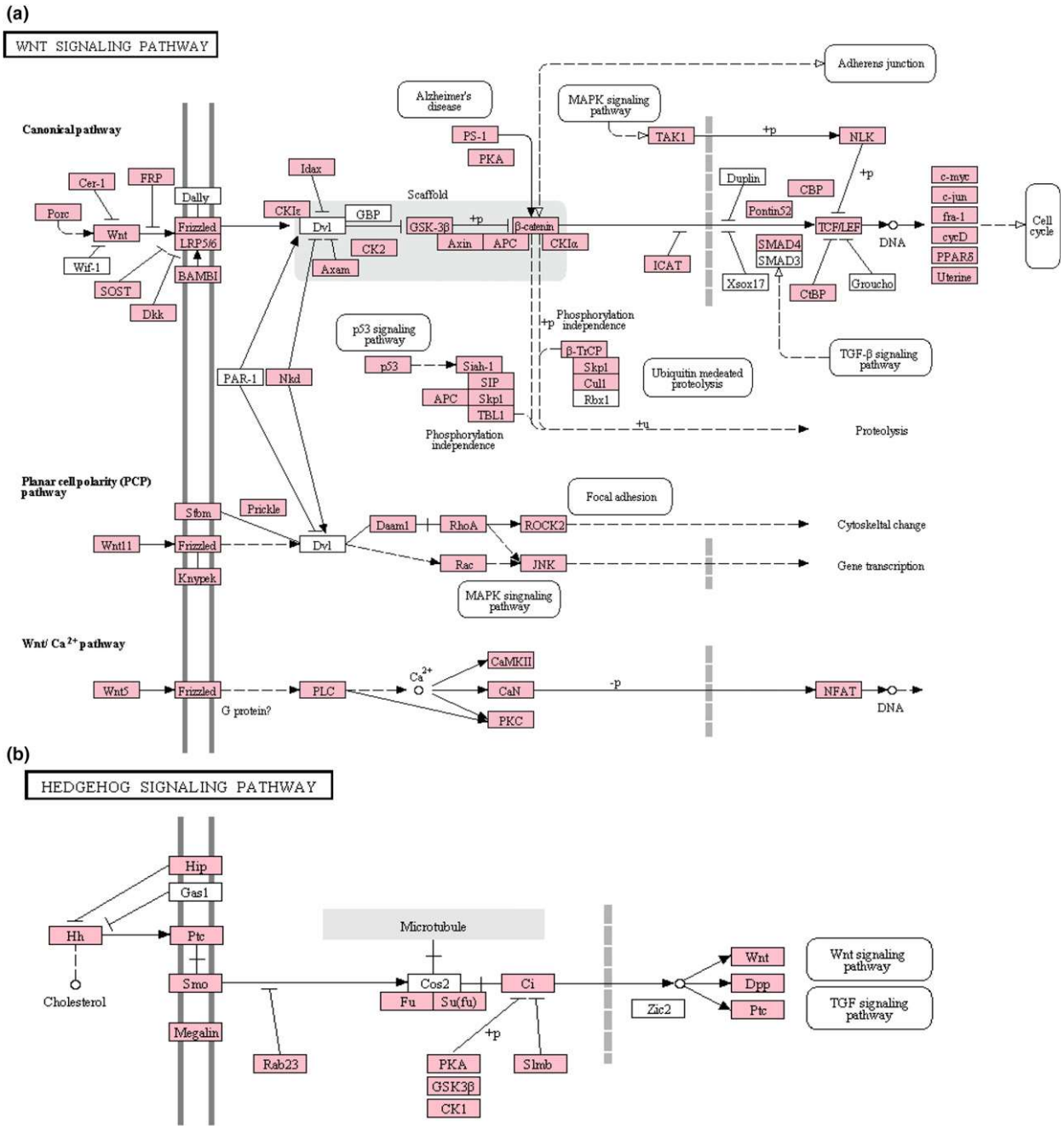

Figure 5 Differentially expressed genes and relative pathways assigned to the (a) Wnt signaling pathway and (b) Hedgehog signaling pathway. Red boxes indicate expressed genes; these two pathways have effects on wool length.

differentiation, morphogenesis of the skin and HF, skin development and hair growth through the Wnt/*β*-catenin signaling pathways (Reddy *et al.* 2001; Xing *et al.* 2013, 2016).

β-catenin signaling is not only involved in the formation of skin epithelial cells but is also closely related to the proliferation and differentiation of skin cells as well as the growth and development of HFs (Andl *et al.* 2002). In addition, the Hedgehog signaling pathway is a key molecular signaling mechanism of skin morphogenesis (Millar 2002; Schmidt-Ullrich & Paus 2005). Hedgehog signaling is required for the cytodifferentiation of follicular keratinocytes (St-Jacques *et al.* 1998; Mill *et al.* 2003; Woo *et al.* 2012). During skin morphogenesis, sonic hedgehog, the predominant Hedgehog ligand in the skin, is produced by follicular keratinocytes and regulates Hedgehog signaling in follicular keratinocytes and DP cells.

Fibroblast growth factors are expressed in numerous species, from nematodes to humans, and play various roles in development and metabolism as well as in the regulation of cell proliferation, migration and differentiation (Itoh & Ornitz 2004). Previous research confirmed that FGF5 is closely related to hair growth (Hébert *et al.* 1994; Chong 2014). FGF5 is a secreted signaling protein belonging to the FGF family of proteins, which are polypeptide growth factors (Eswarakumar *et al.* 2005; Bao *et al.* 2015). As a regulator of hair length, FGF5 has been previously reported in a variety of mammals. Knock-out of FGF5 using the CRISPR/Cas9 system in goat led to an increased number of secondary HFs, and FGF5 could be a key biological factor influencing the HF cycle through effects on the anagen–catagen transition (Higgins *et al.* 2014; He *et al.* 2016; Wang *et al.* 2016). *FGF5* was up-regulated in the L group, indicating that *FGF5* expression has a positive effect on wool growth.

Bone morphogenetic protein 4 is known to be involved in many biological processes and participates in many skin and wool development-related signaling pathways, such as the MAPK, TGF-*β* and Hedgehog signaling pathways. *BMP4* transcript levels are intimately associated with secondary hair follicle development, increasing the quantity and density of follicles (Menzies *et al.* 2009). The interaction between BMP4 and PTHrP inhibits the induction of hair follicle formation, and BMP4 activates the expression of *LEF1* to regulate hair development (Kratochwil *et al.* 1996; Hens *et al.* 2007). In addition, the quiescence of HF is maintained by the BMP signaling pathway, and the expression of *BMP4* is reduced in dermal papillae when the hair cycle begins at telogen (Lee & Tumbar 2012). Moreover, *MSX2* is regulated by BMPs in several developmental processes, leading to the expression of hair matrix transcription factors that promote the proliferation of hair matrix cells at early stages of HF development (Kratochwil *et al.* 1996; Kulessa *et al.* 2000; Towler *et al.* 2006; Hens *et al.* 2007). According to our results, *BMP4* and *MSX2* were highly expressed in rabbits with the long-wool phenotype, demonstrating that expression of these genes may promote HF differentiation and development. Overall, our results are crucial for a thorough understanding of the functions of the abovementioned genes, and their roles in HF development and proliferation in rabbits should be further investigated.

Assessment of gene expression in rabbit breeds

Long-wool phenotype rabbits (Wanxi Angora) and short-wool phenotype rabbits (White Rex and Chinchilla Rex) were used to evaluate the gene expression of *WNT5A*, *FGF5*, *BMP4* and *BMP7*. The expression of all four genes was highest in Wanxi Angora rabbits, followed by White Rex rabbits and Chinchilla Rex rabbits, with no significant difference between White Rex and Chinchilla Rex rabbits (Fig. 6). Previous studies have associated polymorphisms in the *FGF5* coding sequence with the angora phenotype in rabbits, although there is no evidence of complete linkage disequilibrium between any *FGF5* allele and the angora phenotype (Mulsant *et al.* 2004). The transcriptomic analysis showed that *WNT5A*, *FGF5*, *BMP4* and *BMP7* were upregulated in the L group, exhibiting the same gene expression patterns as those in the various long- and short-wool phenotype rabbit breeds. The functional annotation and pathway analysis of these genes reveal their relevance to HF development and hair growth.

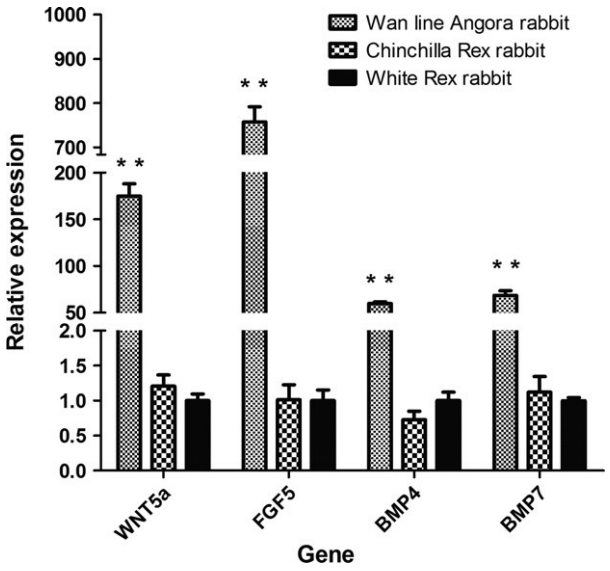

Figure 6 Comparison of candidate gene expression levels in different wool-length rabbit breeds. Gene expression levels of *WNT5A*, *FGF5*, *BMP4* and *BMP7* were determined in Wanxi Angora, White Rex and Chinchilla Rex rabbits using RT-qPCR. The expression levels of genes were normalized to those in White Rex rabbit. Error bars indicate the mean hiS.D. of triplicate experiments. $^{**}P < 0.01$.

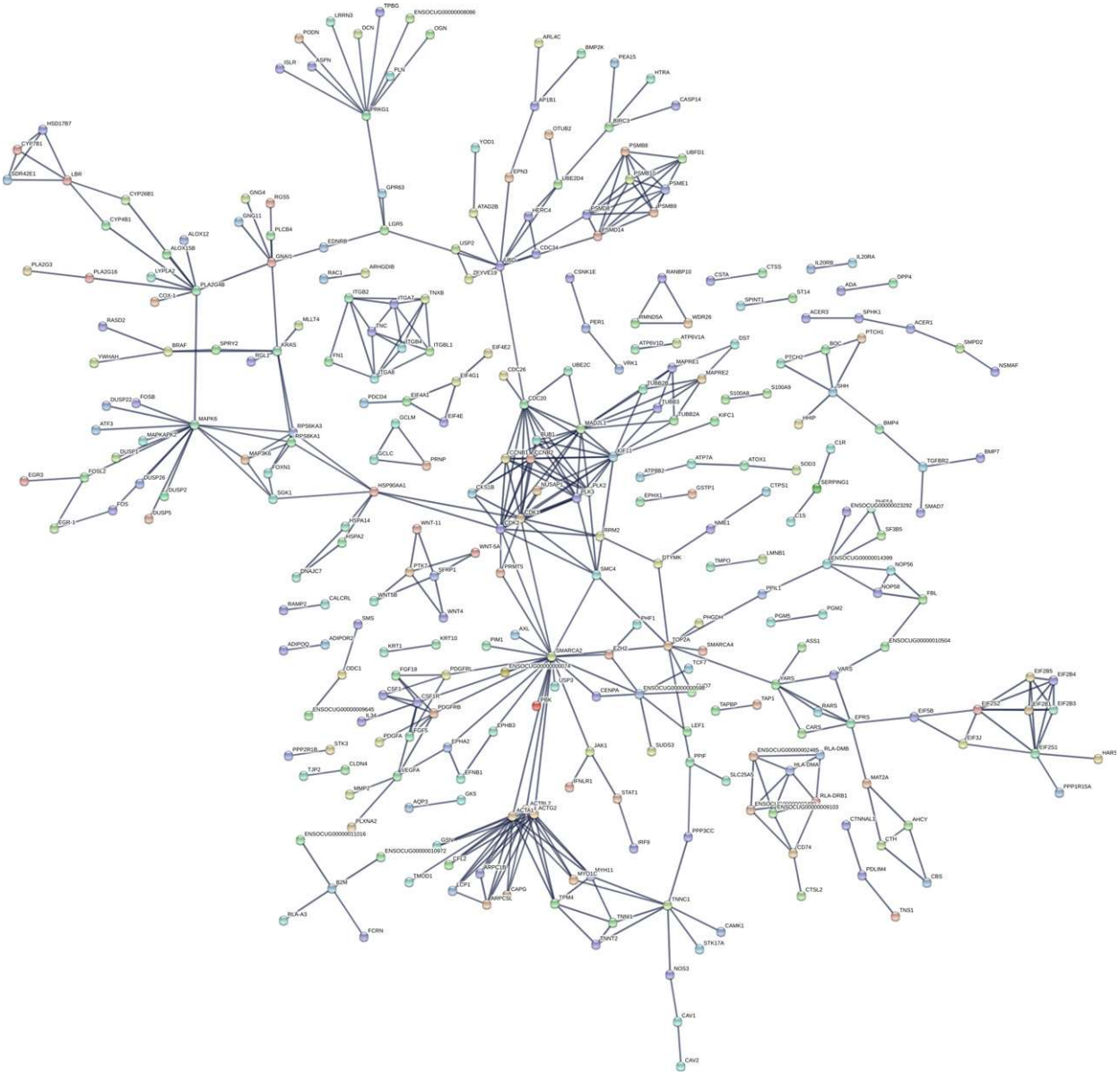

Figure 7 Diagram of the interaction network. Thicker lines represent stronger interactions between differentially expressed genes and their interaction partners. The minimum required interaction score has the highest confidence (0.900).

Network analysis of DEG interactions

To explore interactions among DEGs, the STRING database was used to construct a protein–protein interaction network from active interaction sources, such as text mining, experiments, databases, co-expression, neighborhood, gene fusion and co-occurrence information. The network of DEG interactions shows functional associations among proteins that were identified using DEG interacting partners (Fig. 7). We observed that Wnt family proteins, such as Wnt4, Wnt5a, Wnt5b and Wnt11, were related to PTK7 and SFRP1. As a Wnt co-receptor, PTK7 inhibits the canonical Wnt signaling pathway, and SFRP1 acts an antagonist to modulate the action of Wnt family proteins, regulating cellular activity (Hoang *et al.* 1998; Peradziryi *et al.* 2012; Matsuyama *et al.* 2014). Wnt family proteins play key roles in many biological processes, including HF growth and differentiation, and Wnt proteins are required for initiating HF development (Andl *et al.* 2002; Huang *et al.* 2012).

Conclusion

In this study, we found 17 086 expressed genes, including 1321 DEGs between rabbits with long and short wool. The main GO terms of these genes were related to metabolism, development, regeneration and response to stimuli, which

are vital to the growth of HFs. KEGG analysis showed that a total of 394 DEGs were involved in 241 pathways. The Wnt and Hedgehog signaling pathways are involved in embryonic development and cyclical growth of the HF. Many genes, such as *WNT5A*, *WNT11*, *BMP4*, *BMP7*, *MSX2*, *FGF5*, *PDGFA* and *IGFBP5*, were identified in our study as candidate genes likely to influence wool length. Our results are important for a thorough understanding of the functions of these genes in the future and provide candidate wool-length-related functional genes for further study.

Acknowledgements

This work was supported by the Modern Agricultural Industrial System Special Funding (CARS-43-A-1), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD 2014-134) and the Natural Science Foundation of the Higher Education Institutions of Jiangsu Province (16KJB230001).

Conflict of interest

BoHao Zhao, Yang Chen, Ye Hao, Naisu Yang, Manman Wang, Mengran Mei, Jingyi Wang, Xiaoqing Qiu and XinSheng Wu declare that they have no conflict of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Mapping ratios of reads to each gene region.

Figure S2 GO functional analysis of differentially expressed genes.

Figure S3 Histogram of KEGG pathway functional classifications.

Table S1 Primers used for quantitative real-time PCR analysis.

Table S2 Differentially expressed genes between long wool rabbits and short wool rabbits.

Table S3 GO classifications of differentially expressed genes.

Table S4 KEGG pathway annotations of differentially expressed genes.