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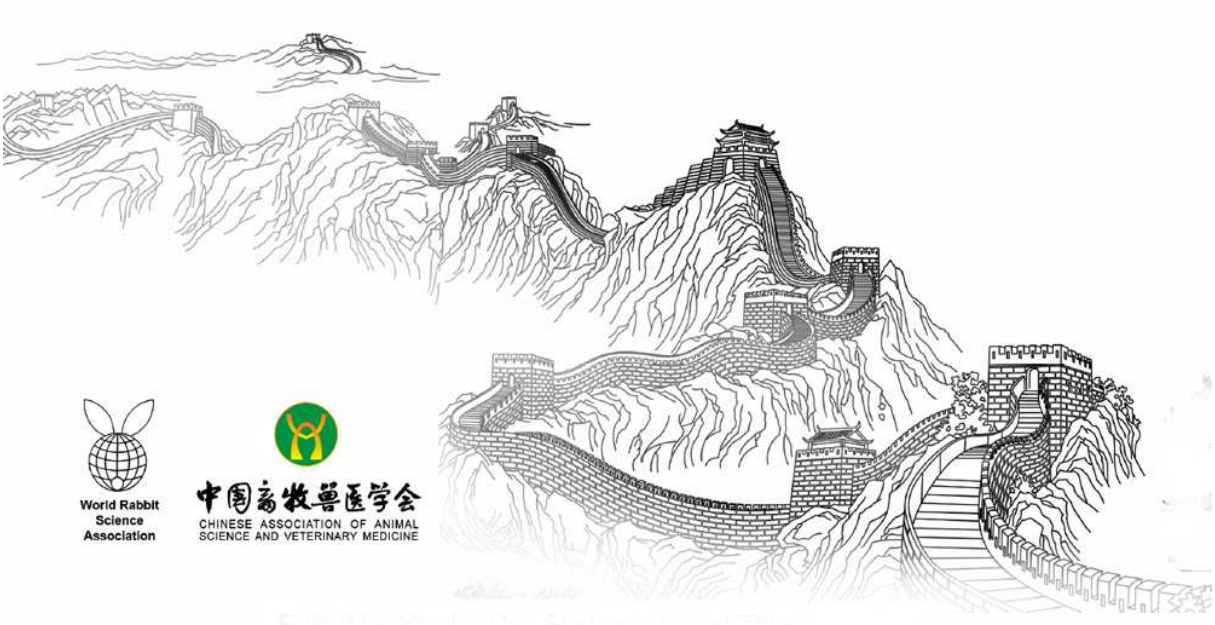

IDENTIFICATION AND DIFFERENTIATION OF TRANSCRIPTOME PROFILES IN CHINCHILLA AND WHITE REX RABBIT SKIN

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ABSTRACT

Prevenient molecular genetic researches of physiology and pigmentation of Rex rabbit skin have focused basically on a scarce number of genes. To identify additional genes that probably play important roles in hair color regulation, high-throughput sequencing technology was used to analyse whole genome expression profiles in skin of Rex rabbit with Chinchilla versus White coat color. Of the 174 known genes differentially expressed in Chinchilla versus White Rex rabbit skin, 101 had a specific KEGG pathway annotation. We found 3 differentially significant (P≤0.01) down-regulation genes of *TYRP1*, *DCT* (*TYRP2*) and *MLANA* (*MART1*) in Chinchilla versus White Rex rabbit skin. There were 2 differentially expressed genes both involved in melanogenesis and tyrosine metabolism (*TRYP1*, *DCT*). These three genes were potentially related to Chinchilla colour formation.

Key Words: Chinchilla,White Rex,Skin, transcriptome profiles, Identification,Differentiation

INTRODUCTION

The Chinchilla Rex rabbit is a breed which has a high commercial value for its fur. Trends that determine hair color in Rex rabbit are becoming of increasing interest. White fur holds greatest economic value due to its ability to be dyed to any color actually, however interest in natural colors is increasing due to the green environmental protection and consumer favorite for genuine products.

Coat color is determined by amounts and types of melanin produced and released by melanocytes resident in the skin (Ito *et al.*, 2000, 2008). The genetic basis for coat color is well understood in rodents, with many common genes also implicated in regulation of coat color in other species, including Rex rabbit (Shi *et al.*, 2015). For example, MC1R and ASIP are known to be major regulators of coat color in mice (Bultman *et al*., 1992; April and Barsh., 2006) and MC1R and ASIP loci are functionally linked to undesirable coat color phenotypes in rabbit (Fontanesi *et al.*, 2010a, 2010b).

Despite considerable knowledge of the genetic regulation of coat color in mice and identification of loci involved in coat color regulation in fiber producing species, the molecular mechanisms, at the level of gene expression, associated with differences in coat color phenotype are not well understood. So we investigated the transcriptome profiles in skin of Chinchilla and White-coat Rex rabbit using high throughput RNA deep sequencing. Results provided novel insight into differences in gene expression associated with coat color, including key genes implicated in the melanogenesis pathway.

MATERIALS AND METHODS

Rex rabbit skin sampling and total RNA extraction

Six healthy 50-day-old Chinchilla and white female Rex rabbit (3 Rex rabbit per color) were selected for sample collection from the Rex rabbit farm of Sichuan Jin Fu modern agricultural limited company in Luojiang county, Deyang city, China. A piece of skin (8 mm in diameter) from the body was collected via punch skin biopsy under local anesthesia and immediately placed in liquid nitrogen.

Total RNA from the sample was extracted using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. The RNA integrity was evaluated by gel electrophoresis and the RNA purity was checked by the ratio of OD260/OD280 and RIN value. RNA samples with RIN value greater than 7.5 and OD260/ OD280 ratio greater than 1.7 were selected for Rex rabbit sequencing.

Library generation and sequencing

Three RNA samples from Chinchilla or white Rex rabbit skins were pooled before mRNA isolation. Beads with Oligo (dT) were used to isolate poly (A) mRNA from Rex rabbit skin total RNA. The isolated mRNA was fragmented followed by first-strand cDNA synthesis using random hexame r-primers. The second-strand cDNA was synthesized using buffer, dNTPs, RNaseH and DNA polymerase I. The short cDNA fragments were purified using QiaQuick PCR extraction kit (Qiagen, USA). The fragment ends were repaired and A tailed followed by ligation to sequencing adaptors. Suitable size fragments were selected following agarose gel electrophor esis and used as templates for PCR amplification. Sequencing of the library was performed using Illumina HiSeq™ 2000.

Quantification of gene expression level

Cuffdiff (v2.1.1) was used to calculate FPKMs of coding genes in each sample (Cole *et al*., 2010). Gene FPKMs were computed by summing the FPKMs of transcripts in each gene group. FPKM means fragments per kilo-base of exon per million fragments mapped, calculated based on the length of the fragments and reads count mapped to this fragment.

Differential expression analysis

Cuffdiff provides statistical routines for determining differential expression in digital transcript or gene expression data using a model based on the negative binomial distribution (Cole *et al*., 2010). For biological replicates, transcripts or genes with an P-adjust < 0.05 were assigned as differentially expressed. For non-biological replicates, with P-adjust < 0.05 and the absolute value of $\log_2$ (Fold change) < 1 were set as the threshold for significantly differential expression.

KEGG enrichment analysis

KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies (http://www.genome.jp/kegg/). We used KOBAS software to test the statistical enrichment of differential expression genes in KEGG pathways.

RESULTS AND DISCUSSION

Differentially expressed genes in Chinchilla versus White Rex rabbit skin

Volcano plot analysis was used to identify statistically differentially regulated genes based on statistical significance (P≤0.05).A gene was considered statistically significant only if it showed at least 1 fold differences with the P≤0.05.We found 174 differential expressed genes (Fig.1), one of 71 were Up-regulation significant genes (red dots), the others of 103 were down-regulation significant genes (blue dots). We found 3 differentially significant (P≤0.01) down- regulation genes of *TYRP1*, *DCT* (*TYRP2*) and *MLANA* (*MART1*) in Chinchilla versus White Rex rabbit skin.

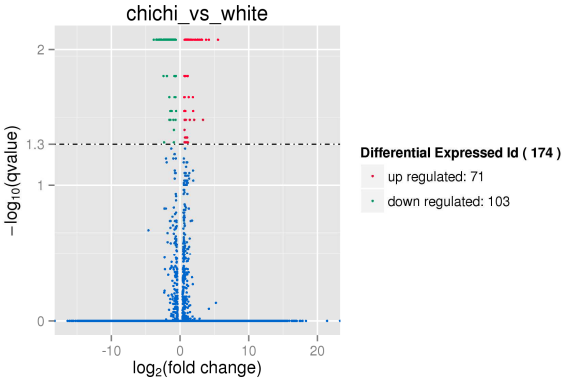

Figure 1 : Volcano plot displayed statistically differentially regulated genes

KEGG pathway analysis

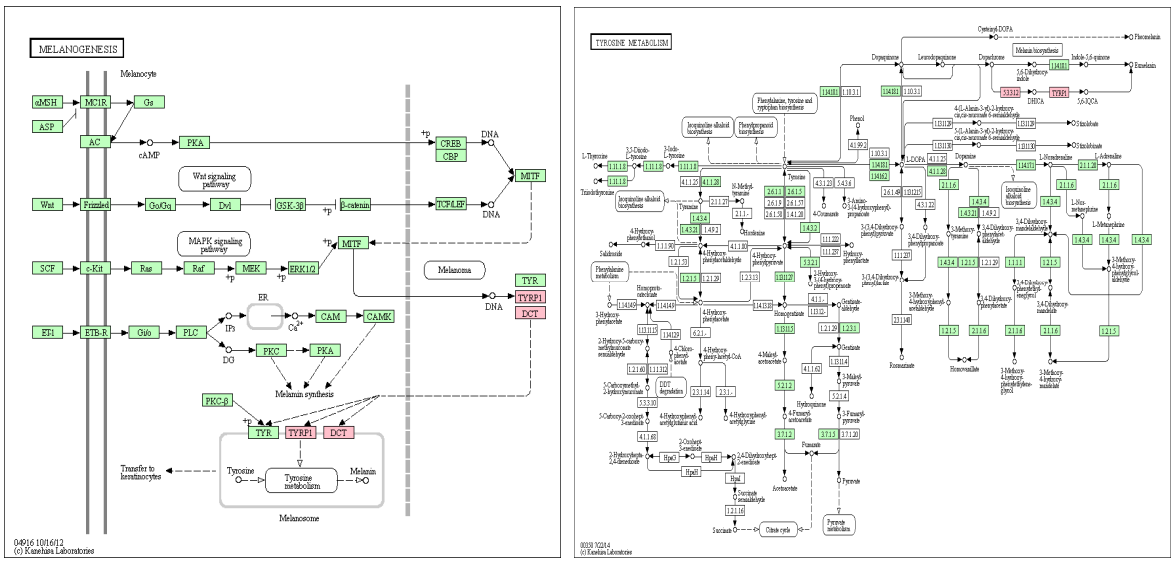

Figure 2: Differentially expressed coat color genes in Chinchilla versus White Rex rabbit skin and their involvement in the melanogenesis (left) and tyrosine metabolism (right) pathway.

Of the 174 known genes differentially expressed in Chinchilla versus White Rex rabbit skin, 101 had a specific KEGG pathway annotation, top 1 KEGG pathway in Chinchilla versus White Rex rabbit skin was Tyrosine metabolism. Of these KEGG pathway annotated genes, KEGG pathway annotated genes were associated with 2 pathways including those functionally related to coat color in skin for melanogenesis and tyrosine metabolism (Fig.2). There were 2 differentially expressed genes (*TRYP1*, *DCT*) both involved in melanogenesis and tyrosine metabolism.

CONCLUSIONS

We found 3 differentially expressed genes of *TYRP1*, *DCT (TYRP2)*, *MLANA* (*MART1*) for coat color, which down-regulation significant genes ($P \leq 0.01$) in Chinchilla versus White Rex rabbit skin. There were 2 differentially expressed genes both involved in melanogenesis and tyrosine metabolism (*TRYP1*, *DCT*). These three genes were potentially related to Chinchilla color formation.

ACKNOWLEDGEMENTS

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