PROCEEDINGS OF THE 11th WORLD RABBIT CONGRESS
Qingdao (China) - June 15-18, 2016
ISSN 2308-1910

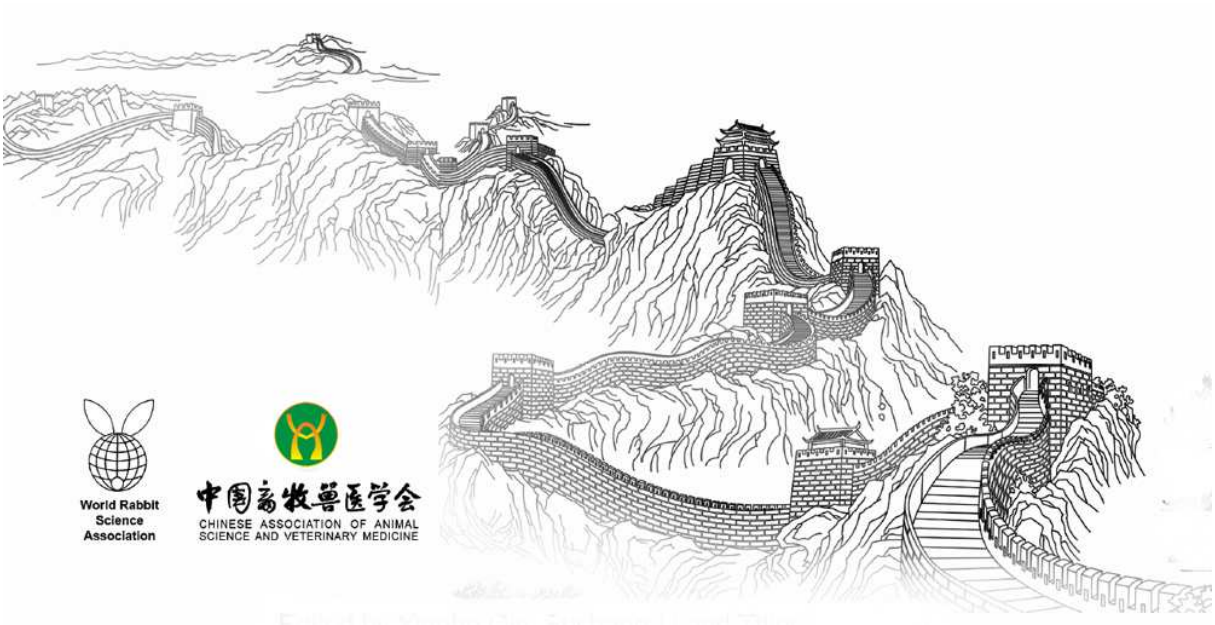
Session
Fur & Wool

Zhao B.H., Chen Y., Yan X. R., Hao Y., Zhu J., Weng Q.Q., Wu X.S.

GENE EXPRESSION ON PROFILING ANALYSIS REVEALS COAT COLOR FORMATION IN REX RABBITS (*Oryctolagus cuniculus*)..

Full text of the communication

How to cite this paper :
Zhao B.H., Chen Y., Yan X. R., Hao Y., Zhu J., Weng Q.Q., Wu X.S., 2016 - Gene expression on profiling analysis reveals coat color formation in rex rabbits (*Oryctolagus cuniculus*).. *Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China, 873-876.*
.



GENE EXPRESSION PROFILING ANALYSIS REVEALS COAT COLOR FORMATION IN REX RABBITS (*ORYCTOLAGUS CUNICULUS*)

Zhao B.H.[1,2], Chen Y.[1,2], Yan X.R.[1,2], Hao Y.[1,2], Zhu J.[1,2], Weng Q.Q.[3], Wu X.S.[1,2]*

[1]College of Animal Science and Technology, Yangzhou University;
[2]Jiangsu Key Laboratory of Animal Genetics & Breeding and Molecular Design, Yangzhou, Jiangsu 225009 China;
[3]Zhejiang Yuyao Xinnong Rabbit Industry Co., Ltd., Yuyao, Zhejiang 315400, China
*Corresponding author E-mail: xswu@yzu.edu.cn

ABSTRACT

Coat color is an important economic trait in rabbit production. The identification of genes influencing coat color formation and knowledge of the actions of these genes in question provide useful tools for improving fur quality. However, the formation mechanism of coat color is unclear. To obtain coat color-related candidate genes, tissues of the backs and bellies of three full-sibling Chinchilla rex rabbits were used to analyze changes in genomic expression by transcriptome analysis. Of the genes studied, 336 genes showed altered expression in the two groups (285 upregulated and 51 downregulated) by the fold-change and Fisher-test precision inspection statistical methods (P≤0.05, fold-change≥2 or ≤0.5). Using GO and KEGG to obtain differential gene enrichment by hypergeometrics, we compared results with the genomic background and found several genes to be involved in many important biological processes. A number of differentially expressed genes were assigned to three GO categories: biological processes (n=563), molecular functions (n=204), and cellular components (n=132). In addition, several signaling pathways related to the formation of coat color were identified, including the MAPK and Wnt signaling pathways, revealing mechanisms of chromogenesis in coat color, such as tyrosine kinase activity, pigmentation, and melanogenesis. The obtained rabbit transcriptome and differentially expressed gene profiling data provided comprehensive gene expression information for CACNG1, CACNB1, CACNA1S, PTPLA, PTP4A3, TTN, DUSP26, EN1, MT3, SLC25A4, SLC16A3, SFRP2 and FRZB. To validate the RNA-seq data, eight differentially expressed genes related to coat color were confirmed by qRT-PCR. The results of rabbit transcriptome profiling provide a basis for understanding the molecular mechanisms of coat color formation.

Key words: Chinchilla rex rabbit, coat color, gene, transcriptome.

INTRODUCTION

The Chinchilla rex rabbit is an important type of rabbit that has a type of coat colors that grows naturally; consumers highly appreciate the properties of rex furs, such as beauty, softness, colorful, lightness, and warmth retention (Pan et al., 2015). Also, Chinchilla rex rabbits have unique characteristics of coat color, including a sparkling mix of pearl and black, slate blue on the back, and a white and pale blue belly color. The mechanism of coat color regulation is still unclear, so Chinchilla rex is regarded as a model animal to explore the formation mechanism of coat color. Gene expression profiling is an important method for genomic study and identification of functional genes; therefore, we obtained candidate genes using this method and assessed the biological functions.

In this study, we selected coat color-related candidate genes by RNA-seq. The results obtained serve to improve our understanding of the formation of coat color and coat color candidate genes enable us to clarify the mechanisms of coat color formation and provide a valuable theoretical basis for further research of the hair and fur colors of animals.

MATERIALS AND METHODS

Tissue collection, RNA extraction, cDNA library construction and Illumina sequencing

Three healthy, 20-day-old full-sibling rabbits with the same coat color were evaluated. Fur on the back (B group) and belly (F group) were of different coat colors (Figure 1). Two groups have three biological reduplications and it could ensure same genetic background and color phenotype of each group. After transfer to the laboratory, skin tissue samples (1.5 cm^2) were collected from the rabbit's back and belly.

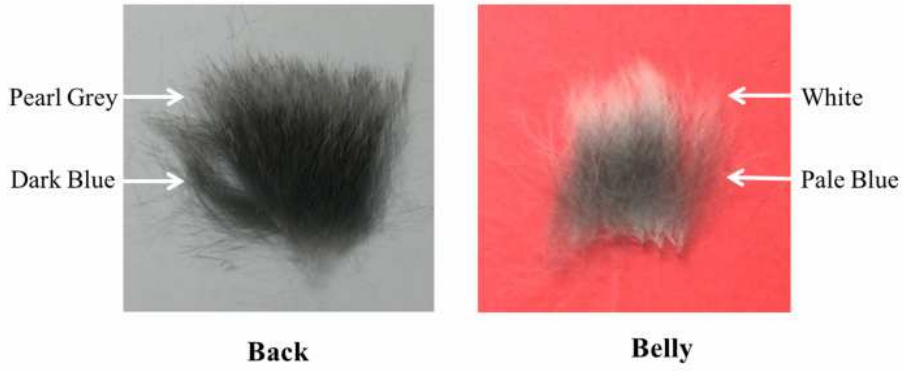


Figure 1: Coat color on the back (left) and belly (right) of a Chinchilla rex rabbit. The rabbit's back fur was dark blue at the base and pearl grey on top, whereas the fur on the belly is pale blue on the lower region and white on the upper region.

Total RNA was extracted and the integrity of the RNA was checked for a RNA Integrity Number, After RNA extraction and purification, 3 μg RNA was used for back and belly cDNA library construction. We depleted the rRNA from the total RNA and then fragmented the RNA. The sample library was completed by cluster generation and cDNA library was sequenced using the Illumina HiSeq 2500 platform (Illumina, San Francisco, USA) and the data were analyzed in real time. We then used the TopHat method (version: 2.0.9) algorithm to map the clean reads to the *Oryctolagus cuniculus* genome by spliced mapping, according to Ensembl OryCun2.0. The quantity of gene expression was calculated using Cufflinks (version: 2.1.1).

Analysis of differentially expressed genes, gene annotation, network analysis and quantitative real-time PCR confirmation of Illumina sequencing data

The Fold-change and Fisher-test precision inspection statistical methods were used to analyze differentially expressed genes that were selected using a false discovery rate control (FDR) less than 0.05 and a fold-change greater than or equal to 2 or less than or equal to 0.5. The differentially expressed genes obtained from the two groups were used for functional annotation and mapped to Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways to identify potential pathways associated with coat color. In addition, P values less than or equal to 0.05 and FDR less than or equal to 0.05 were considered statistically significant. The STRING database was used to perform the network analysis and union of all differentially expressed genes between the two groups were used to build the network. To validate the Illumina sequencing data, eight known candidate genes that significantly differentially expressed between the back and belly were selected for validation by qRT-PCR and the quantitative variation and calculated the relative fold-change based on the $2^{-\Delta\Delta Ct}$ method.

RESULTS AND DISCUSSION

RNA-seq generated clean reads after trimming and clean ratio of greater than 85% for each sample, the total clean reads mapped to the rabbit genome had mapping ratios greater than 80% in this study. 336 genes that were significantly differentially expressed between the back and belly regions of the rex rabbits were identified using fold-change greater than or equal to 2 or less than or equal to 0.5 and FDR less than or equal to 0.05 (285 upregulated genes and 51 downregulated genes). Melanoma is a malignant tumor of melanocytes (Burgic et al., 2010). Previous studies have revealed that overexpression of PTP4A3 in transformed uveal melanocytes results in differential color expression (Laurent et al., 2010). While

DUSP26 absent in human melanoma, occurs in primary human glioblastoma tumors with loss of expression (Patterson et al., 2010). TTN mutations have been identified in multiple cancer types including melanoma (Balakrishnan et al., 2007). Furthermore, EN1 may regulate the embryonic expression of agouti, as shown by analysis of the distribution of agouti transcripts in mice with targeted disruptions (Millar et al., 1995).

Table 1. Primer sequences used in qRT-PCR for the validation of differentially expressed genes.

Gene	Primers
GAPDH	Forward primer: 5'-TCACCATCTTCCAGGAGCGA-3'
	Reverse primer: 5'- CACAATGCCGAAGTGGTCGT-3'
FRZB	Forward primer: 5'-CATCAAGTACCGCCACTCGT-3'
	Reverse primer: 5'-GCCCCTCTACAGTTTCCATTGCT-3'
SFRP2	Forward primer: 5'-CCAGCCCGACTTCTCCTACAAGC-3'
	Reverse primer: 5'-TCCAGCACCTCTTTCATGGTCT-3'
EN1	Forward primer: 5'-CTCCTGGGGCTTATCCGTCC-3'
	Reverse primer: 5'-CTCCCAGTTCCAGCCAAGGTC-3'
CACNA1S	Forward primer: 5'-TCATCCTCAGCGAGATCGACAC-3'
	Reverse primer: 5'-GATCAGCCTCATGACCCGGAAC-3'
DUSP26	Forward primer: 5'-TAACTGGCTCTGGGCATCCAT-3'
	Reverse primer: 5'-CCGCTCCAGCTCGAAGACGTT-3'
PTP4A3	Forward primer: 5'- AGAACATGCGCTTCCTCATCACC-3'
	Reverse primer: 5'-TGTCGTAGGTCACTTCGCACAC-3'
HBB1	Forward primer: 5'-GCTGCTGGTTGTCTACCCAT-3'
	Reverse primer: 5'-AGCCAGCACCTTCTTGCCAT-3'
MRPL36	Forward primer: 5'-CCCGCGCTGGGCTTCAAGAC-3'
	Reverse primer: 5'- GGGTTGCTCTCGCAGTACACGAAC-3'

According to the GO analysis, categories of biological process (n=563), molecular function (n=204), and cellular component (n=132) contained 214 differentially expressed genes, which were all significant. GO analysis revealed that the majority of differentially expressed genes were associated with protein tyrosine phosphatase activity, protein tyrosine kinase activity, and protein tyrosine phosphatase activity. These genes could regulate pigmentation by altering protein tyrosine phosphatase activity, protein dephosphorylation, protein phosphorylation, and protein kinase activity. Meanwhile, we obtained 66 differentially expressed genes by KEGG pathway analysis, for example, cardiac muscle contraction, dilated cardiomyopathy, and adrenergic signaling in cardiomyocytes. Analyzing all KEGG signaling pathways, there was a definite relationship between several pathways, such as the Wnt and MAPK signaling pathways. Our study found color-related genes in signaling pathways participate in melanogenesis, such as the MAPK and Wnt signaling pathways, which are involved in melanogenesis through the MITF, which primarily regulates melanocyte development by modulating differentiation and cell cycle progression (Russo et al., 2009).

To explore the interactions of differentially expressed genes, we used RNA-seq data to construct a differentially expressed gene interaction network. We identified the interactions of differentially expressed genes using the STRING database, which predicts functional associations between proteins. To confirm our results, qRT-PCR was performed and we found that FRZB, SFRP2, DUSP26, PTP4A3, EN1, and CACNA1S were upregulated, and HBB1 and MRPL36 were downregulated. These data indicate that the results from transcriptome study are consistent with the overall changes of differentially expressed genes.

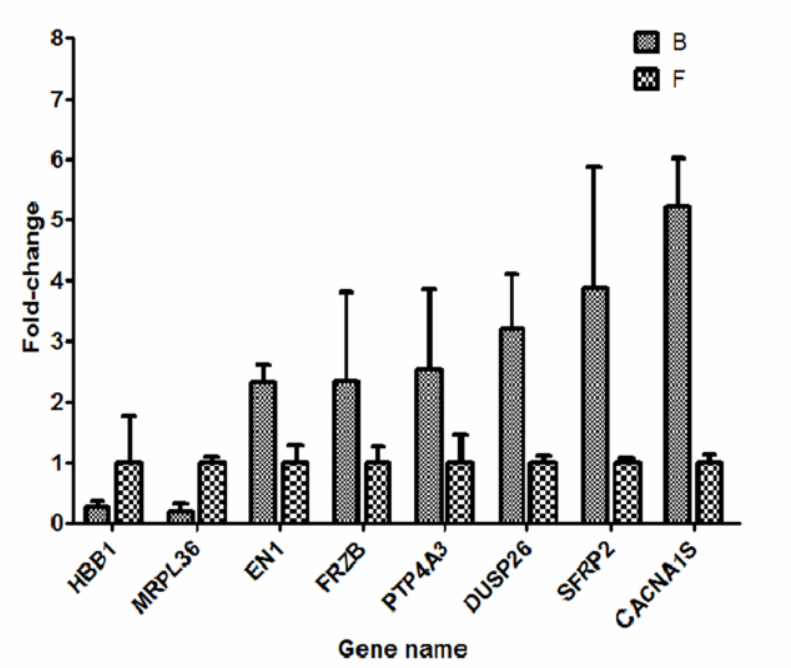


Figure 2: Analysis of differentially expressed genes involved in regulation of coat color in rabbits by qRT-PCR analysis. Gene expression levels between back group (B) and belly group (F).

CONCLUSIONS

Gene expression profiling analysis was used to assess coat color of Chinchilla rex rabbits. This study found several genes associated with coat color, including CACNG1, CACNB1, CACNA1S, PTPLA, PTP4A3, TTN, DUSP26, EN1, MT3, SLC25A4, SLC16A3, SFRP2, and FRZB, which affect pigmentation in rabbit skin. The mechanisms regulating coat color are complex, and coat color-related genes should be studied further using various methods.

ACKNOWLEDGEMENTS

This work was supported by the Modern Agricultural Industrial System Special Funding (CARS-44-1) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (2011-137).

REFERENCES

Balakrishnan A., Bleeker F.E., Lamba S., Rodolfo M., Daniotti M., Scarpa A., van Tilborg A.A., Leenstra S., Zanon C. & Bardelli A.. 2007. Novel somatic and germline mutations in cancer candidate genes in glioblastoma, melanoma, and pancreatic carcinoma. *Cancer research. 67(8):3545-50.*

Burgic M., Rifatbegovic A., Hadzigrahic N. & Halilbasic E. 2010. Clinical and Morphological Characteristics of Skin Melanoma: a 5 Year Retrospective Study. *Acta Medica Saliniana. 39(1).*

Laurent C., Couturier J., Sastre-Garau X., Desjardins L., Barillot E., Piperno-Neumann S. & Saule S. 2010. PTP4A3, a Signal Molecule Deregulated in Uveal Melanoma Metastasis. *Signal Transduction in Cancer Metastasis: Springer. P, 255-63.*

Millar SE, Miller MW, Stevens ME, Barsh GS. 1995. Expression and transgenic studies of the mouse agouti gene provide insight into the mechanisms by which mammalian coat color patterns are generated. *Development. 121(10), 3223-32.*

Pan L., Liu Y., Wei Q., Xiao C., Ji Q., Bao G. & Wu X. 2015. Solexa-Sequencing Based Transcriptome Study of Plaice Skin Phenotype in Rex Rabbits (Oryctolagus cuniculus). *Plos One. 10.*

Patterson K.I., Brummer T., Daly R.J. & O'Brien P.M. 2010. DUSP26 negatively affects the proliferation of epithelial cells, an effect not mediated by dephosphorylation of MAPKs. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 1803(9), 1003-12.*

Russo A.E., Torrisi E., Bevelacqua Y., Perrotta R., Libra M., McCubrey J.A., Spandidos D.A. & Stivala F.,Malaponte G.. 2009. Melanoma: molecular pathogenesis and emerging target therapies (Review). *International journal of oncology. 34(6), 1481-9.*

==========