MICROARRAY ANALYSIS OF GENE EXPRESSION PROFILES IN REX RABBIT SKIN

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ABSTRACT

In order to identify differentially expressed genes in Rex rabbit skin, the expression of all genes in the skin and hair follicle of Rex rabbit were analyzed using cDNA microarray, and then analyzed the differently expressed genes with the Gene Ontology (GO) classification and the pathway analysis. The 2657 differentially expressed genes were identified. Among differentially expressed genes, 1103 genes were functionally known genes, 687 genes were up-regulated and 419 down-regulated. GO analysis indicated that these altered genes were associated with metabolism, signal transduction, cell cycle, cell adhesion, cell proliferation, cell division, apoptosis and other processes. KEGG analysis showed that 95 signal pathways associated with up-regulated genes and 87 signal pathways associated with down-regulated genes had changed significantly ($P<0.05$). Some important genes were identified, which might be useful in further study on wool density markers of Rex rabbit.

Key words：Rex rabbit, gene expression, cDNA microarray, GO and KEGG analysis.

INTRODUCTION

Rex rabbit is a typical rabbit for fur. Wool density, hair length, coarse rate, villus diameter and area of hide are main indices of fur quality (Castle *et al*. 1933; Wang *et al*. 2001). The current problem of rabbit fur quality is mainly focusing on the wool density and area of hide. How to improve the quality of our Rex rabbit's fur in order to enhance market competitiveness is a critical problem.

It is well-known that tissues from specific types or phenotype will have distinct gene expression profiles (Wang et al. 2005; Li et al. 2008; Maaria et al. 2010). cDNA microarrays enable the simultaneous measurement of expression levels for many thousands of genes at a given instant in hair follicles, revealing the expression state of those features as determined by statistical significance inference. By taking many such snapshots over a number of experimental conditions and applying the appropriate analyses, groups of genes with similar expression profiles can be detected to suggest the function or regulation of uncharacterised genes (Breitling et al. 2004). Gene Ontology (GO) and pathway analysis were used to find

biological processes associated with the differentially expressed genes(Cristina et al. 2005). Through studying the molecular mechanism in different wool density, we can identify some important genes, which may be candidate genes to detect molecular markers linked to wool density by polymorphism analysis and hence helpful for the early selection-breeding of Rex rabbit.

In this study, the gene expression patterns in different wool density of Rex rabbit are presented and analyzed with cDNA microarray and statistical analysis.

MATERIALS AND METHODS

Preparation of animals and tissue samples

This experiment was carried out at the Rex rabbit farm of Jun-ying, Bao ding, China. All of Rex rabbits were born in 19 September 2009. The fur production traits (wool density, hair length) and live body weight observed at 14 weeks of age. They were divided into 2 groups according to different wool density, group A: The hair is sparse; group B: The hair is thick. In each group, 4 rabbits (2 males and 2 females) were selected. The selection criteria is that wool density is around 10000 per square centimeter in group A and around 18000 per square centimeter in group B. The mid-dorsal skin samples obtained from 8 Rex rabbits were snap-frozen and stored in liquid nitrogen until use.

Gene expression analysis by cDNA microarray

Total RNA from skin tissue samples was isolated by the guanidinium isothiocyanate method. Concentration and RNA quality were assessed via spectrophotometry and denaturing agarose gel electrophoresis. Divided the mRNA of each rabbit into two lots. Then the RNA was used as template for cDNA synthesis for 2h at 42□. All kinds of RNA were labeled with Cy5-dCTP, respectively. The remaining group A's RNA was mixed and labeled with Cy3-dCTP as common reference.

The labeled cDNA samples were separated from unincorporated nucleotides by filtration, mixed and hybridized to microarray at 42□ overnight. After hybridization, the slide was washed, and then scanned with LuxScan 10KA(CapitalBio Corporation). Primary data collection and analysis were carried out using LuxScan 3.0 and SAM 3.02(CapitalBio Corporation).

Statistical analysis of microarray data

The relative levels of gene expression is group B compared with group A. Genes with average expression intensity ratios, higher than 2.0 or lower than 0.5, were analyzed with hierarchical clustering. The analysis was performed using "Cluster 3.0", "TreeView" and Molecule Annotation System (MAS) (Wang *et al*. 2009). Before the clustering, algorithm was applied, the fluorescence ratio for each spot was first log-transformed ($\log_2$), and then the data for each sample were median-centered to remove experimental biases.

RESULTS AND DISCUSSION

Differentially expressed genes by cDNA microarray

In the gene-chip experiment, 2657 differentially expressed genes with average Cy5:Cy3 ratios higher than 2.0 or lower than 0.5 were identified from 14601 target genes. Among them, 1103 genes were functionally known genes. 687 genes were up-regulated and 419 down-regulated.

Gene Ontology Classification and the pathway analysis

To categorize the differentially expressed genes, we performed GO and KEGG analyses. GO annotations were collected for the 1103 selected genes, downstream molecules corresponding to most of the EST probe sets were neither annotated nor included in the analyses. GO analysis indicated that these altered genes were associated with metabolism, signal transduction, cell cycle, cell adhesion, cell proliferation, cell division, apoptosis and other processes.

KEGG analysis showed that 95 signal pathways associated with up-regulated genes had changed significantly ($P<0.05$), these pathways were involved in focal adhesion, TGF-beta signaling pathway, cell adhesion molecules, MAPK signaling pathway, Wnt signaling pathway and so on(Figure 1); 87 signal pathways associated with down-regulated genes had changed significantly ($P<0.05$), those pathways were involved in cell cycle, oxidative phosphorylation, regulation of actin cytoskeleton, p53 signaling pathway and so on (Figure 2).

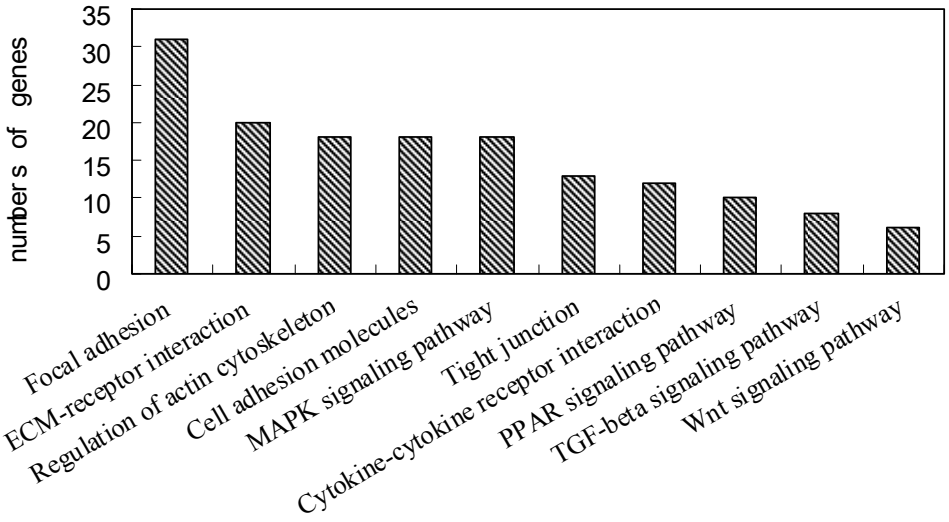

Figure 1: A part of KEGG pathways associated with up-regulated genes

Some potentially important genes in different wool density of Rex rabbit were identified, such as MMP2, TGF-β1, TGF-β2, IGF-1, ITGB1, RPS6KB1, BMP2, ActR□B, CDK2 and CCNA2. Compared with group A, the expression of cell adhesion molecules such as integrin β, L-selectin, ITGB1, and FN were up-regulated at group B. Apart from increased expression of TGF-β1, TGF-β2, TGF-induced protein, IGF-1, SLC, EF-1, and CTGF, all other cytokines and growth factors exhibited decreased expression at group B. BMP2, CDK2, GJA1, CCNA2, ActR□B, RGN, RPS6KB1, which function in cell cycle control and cell signaling, were up-regulated at group A. Whereas some other

related molecules, such as caveolin, YB-1, and TMEM 109, were up-regulated at group B. MMPs and their inhibitor molecules were differentially expressed in different wool density of Rex rabbit. Group B were found to have higher expression of MMP2, TIMP-1, TIMP-2, and TIMP-3 and lower expression of MMP-12 compared with group A. Urokinase-type plasminogen

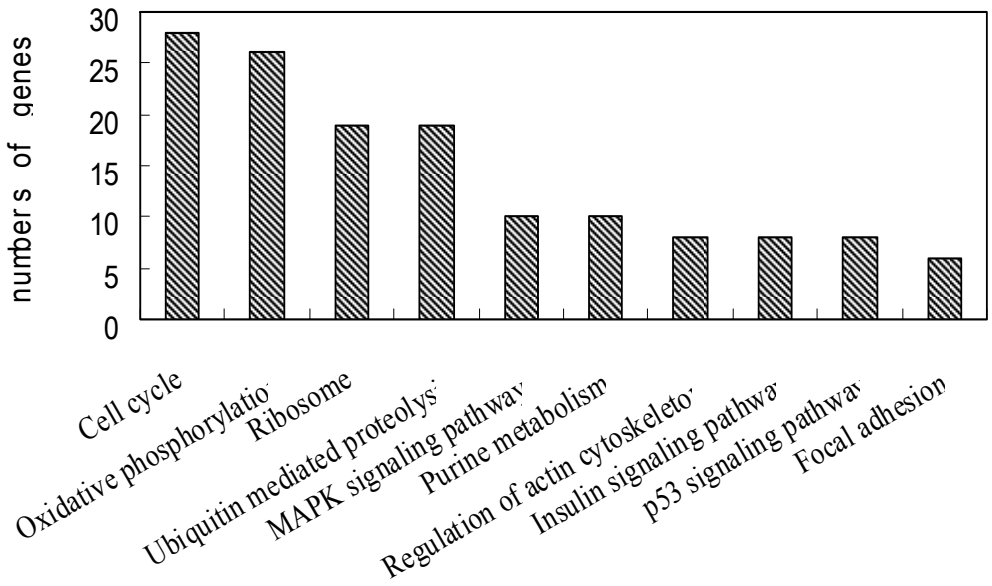

Figure 2: A part of KEGG pathways associated with down-regulated genes

activator receptor was up-regulated in group B. Many of the enzymes were up-regulated at group A. Most transcription factors and structural genes showed augmented expression at group B. Among the classical signaling pathways, expression of BMP2 were up-regulated at group A, and most other signaling pathways were predominantly down-regulated at group A.

The study indicated that the differentially expressed genes were associated with metabolism, signal transduction, cell cycle, cell adhesion, cell proliferation, cell division and apoptosis. The cell cycle, or cell-division cycle, is the series of events that takes place in a cell leading to its division and duplication (replication). The cell-division cycle is a vital process by which a single-celled fertilized egg develops into a mature organism, as well as the process by which hair, skin, blood cells, and some internal organs are renewed. TGF-beta signaling pathway is involved in many cellular processes in both the adult organism and the developing embryo including cell growth, cell differentiation, apoptosis, cellular homeostasis and other cellular functions (Lefever et al., 2010; Plasari et al., 2010). The MAPK/ERK pathway is a signal transduction pathway that couples intracellular responses to the binding of growth factors to cell surface receptors, participate in the regulation of cell proliferation, cell differentiation and apoptosis (Lu et al., 2009). Wnt signaling pathway has promotion in cell adhesion and differentiation (Staal et al., 2008; Kanwar et al., 2010). p53 signaling pathway is a transcription factor whose activity is regulated by phosphorylation. It could be involved in the apoptotic pathway (Cho et al., 2010).

CONCLUSIONS

In this research, the 2657 differentially expressed genes in different wool density of Rex rabbit were identified by using cDNA microarray. Among differentially expressed genes, some genes were related to cell proliferation, differentiation, apoptosis and signal transduction. The abnormal expression of these genes may play an important role in the hair follicles initiation and development, finally lead to the distinction in wool density of Rex rabbit. Some differentially expressed genes may be candidate genes to detect molecular markers linked to wool density by polymorphism analysis and hence helpful for the early selection-breeding of Rex rabbit.

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