Transcriptome analysis of skin color variation during and after overwintering of Malaysian red tilapia

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Background

Tilapia is one of the excellent fish species recommended by the Food and Agriculture Organization of the United Nations. In recent years, tilapia has been widely accepted and has become an export dominant species of aquaculture in China. Red tilapia is a valuable fish due to its uniform red skin, the absence of black peritoneum, very fast growth and adaptability to any culture system, and it has a huge market in many parts of the world, such as China, Malaysia and Thailand. However, the key issue restricting the growth of commercial red tilapia cultures is skin color variation during overwintering. And skin color variation during overwintering period is reversible with the environmental temperature increasing. In this study, three types skin of red tilapia, including the skin remained pink color during and after overwintering (WP), the skin changed from pink color to black color during overwintering and remained black color after overwintering (PB), and the skin changed from pink color to black color during overwintering but recovered to pink color when the temperature rose after overwintering (BP), were used to analyze their molecular mechanisms of color variation.

Experiment

In this study, we used RNA-Seq to analyze the transcriptional profiles of WP, PB and BP skin color of red tilapia during and after overwintering. Particularly, we attempted to screen hundreds of differentially expressed genes (DEGs), which were responsible for skin color variation. Furthermore, the signaling pathways related to color variation during and after overwintering were also examined. Finally, several DEGs were validated by quantitative real-time polymerase chain reaction (qRT-PCR). These findings will help us learn more about the molecular mechanism of skin pigmentation in red tilapia. More specially, it provides valuable genetic data for breeding improved red tilapia strains with consistent skin color.

Result & Discussion

Table 1 The specific statistics for each library sequencing and quality control

Sample	Raw reads	Raw base(G)	Clean reads	Clean base(G)	Q30(%)	GC(%)
BP-1	40,313,772	6.05	40,254,280	6.00	95.23	48.31
BP-2	44,376,218	6.66	44,317,948	6.61	95.01	47.34
BP-3	40,589,108	6.09	40,530,756	6.05	95.01	47.84
BP-4	45,606,884	6.84	45,553,738	6.80	95.12	48.52
PB-1	46,324,834	6.95	46,289,448	6.88	93.32	47.41
PB-2	43,749,772	6.56	43,722,494	6.49	93.97	48.04
PB-3	40,425,896	6.06	40,402,734	5.98	93.81	48.37
PB-4	45,185,008	6.78	45,138,356	6.71	94.34	47.72
WP-1	43,218,496	6.48	43,170,434	6.42	93.80	49.02
WP-2	43,187,756	6.48	43,150,158	6.42	93.63	49.12
WP-3	43,362,090	6.50	43,324,748	6.46	93.92	48.15
WP-4	43,776,672	6.57	43,744,596	6.51	94.04	48.86
Average	43,343,042	6.50	43,299,974	6.44	94.27	48.23

After filtering the low-quality reads and removing adaptor sequences, the average of 42,664,181, 43,888,258 and 43,347,484 clean reads were retrieved for further analysis. The percentage of G + C content and Q30 ratio was an average of 48.23% and 94.27%, indicated a high-quality sequence.

Table 2 Ten most abundant genes of three difference colors in red tilapia during and after overwintering

WP	PB	BP
granulin	*tyrp1b*	*baxa*
tat	*cavin2a*	*si:ch73-86n18.1*
ENSONIG00000007142	*zgc:101810*	*crtac1a*
oca2	*pmelb*	ENSONIG00000040753
ENSONIG00000040391	*tyr*	*sdhb*
oni-mir-24a-4	*myh9a*	*ppdpfa*
col10a1a	ENSONIG00000019137	*loxa*
ENSONIG00000039537	*aqp3*	ENSONIG00000009288
slc45a4	*pmela*	ENSONIG00000018504
map3k7cl	*tyrp1a*	*atg7*

Granulin and *tat* gene were abundant from WP skin .*Tyrp1b* gene was the most abundant in PB skin. And *baxa* gene showed dominantly expression in BP skin.

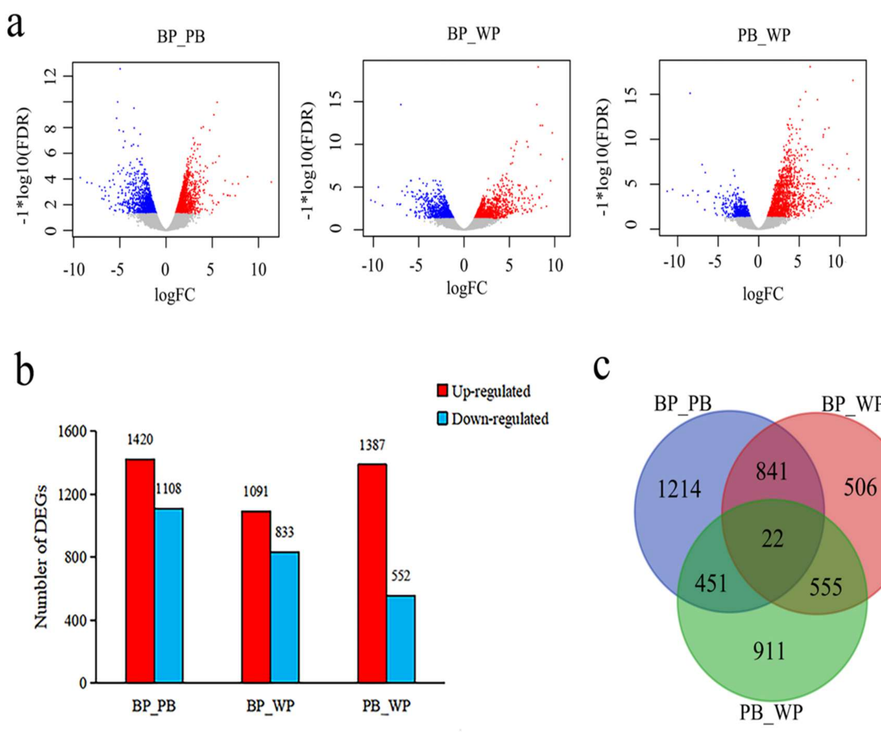

Fig. 1 DEGs in BP, WP and PB skins. a, Volcano plot of differential mRNA expression levels among the three pairwise comparisons. The gray, red, and blue dots represent non-significant, up-regulated and down-regulated transcripts, respectively; b, Number of DEGs among the three pairwise comparisons. The red and blue color stand for up-regulated and down-regulated expression, respectively; c, DEGs number and Venn diagram of the overlap of the different groups.

Under the criteria of FDR ≤ 0.05 and |logFC| ≥1, the volcano plots of three pairwise comparisons (BP_PB, BP_WP and PB_WP) revealed the expression trend of each pair (Fig. 1a). A total of 2,528, 1,924 and 1,939 transcripts were differentially expressed between BP and PB, BP and WP, PB and WP, respectively. (Fig. 1b). Among these genes, 22 DEGs were detected as shared genes in each comparison group, in which 11 were known DEGs and seemed to play a key role in the color variation process (Fig. 1c).

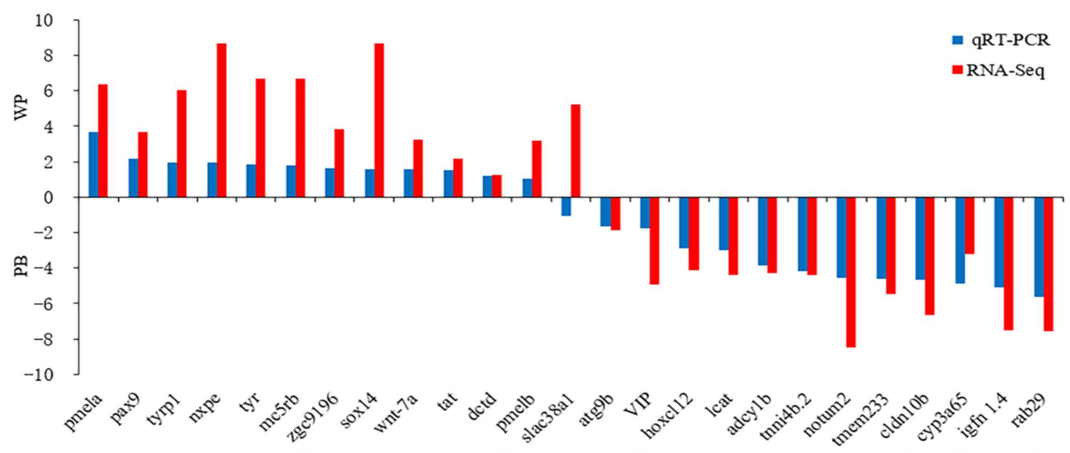

Fig. 2 Comparison of mRNA expression levels among the 25 DEGs obtained using qRT-PCR validation and RNA sequencing. Log-fold changes are expressed as the ratio of gene expression after normalization to *β-actin*

The expression patterns of all down-regulated genes were consistent with the sequencing result, and 12 of the 13 up-regulated genes expression patterns were consistent with the sequencing results. The results showed that the reliability of the sequencing result was high.

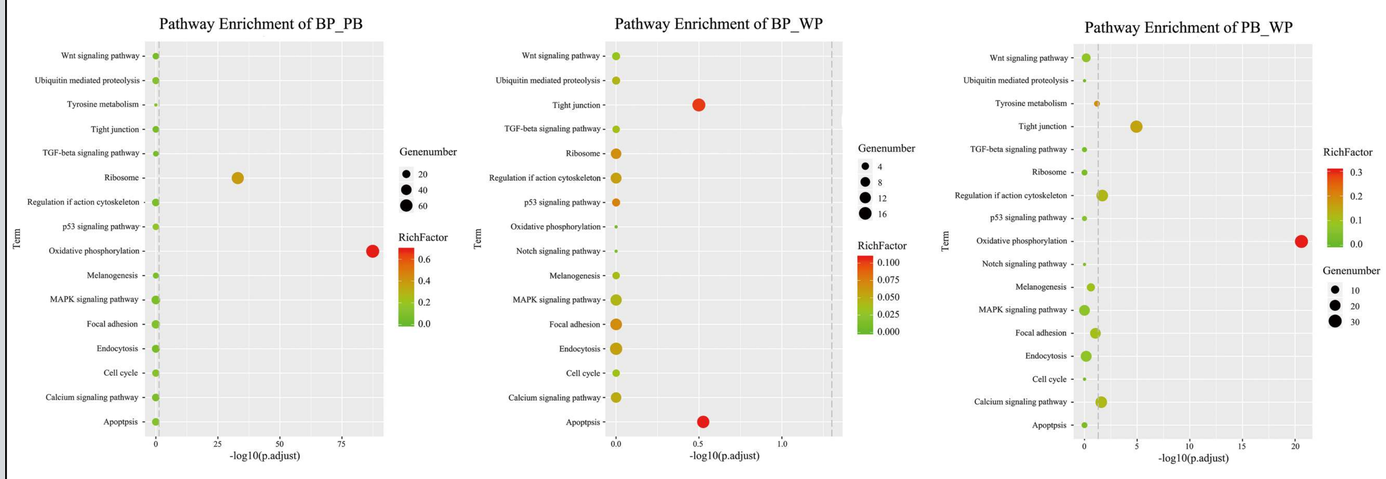

Fig. 3 Pigmentation-related pathways based on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Gene number: number of genes in each pathway; Rich factor: ratio of the number of target genes divided by the total number of genes in each pathway

Several pathways including oxidative phosphorylation, ribosome, Wnt signaling pathway, MAPK signaling pathway, melanogenesis, tyrosine metabolism, autophagy pathway and apoptosis pathway, etc. were identified, which were related to the skin color regulation and pigmentation.

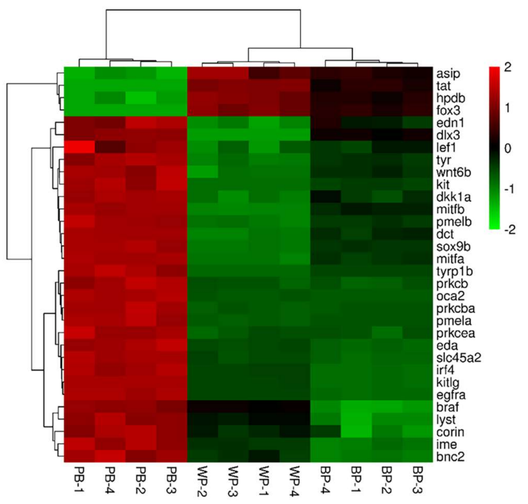

Fig. 4 Heatmap of showing the expressions of selected DEGs in WP, PB and BP skins. Note: Each row in the map represents a DEG and column represents condition used; Log10 normalized expression value is used for constructing heat-map.

Four pigment-related genes including *asip*, *tat*, *hpdb*, and *foxd3* were up-regulated in WP and BP skins compared with the PB skin, while the rest pigment-related genes including *tyr*, *tyrp1*, *mc1r*, *mitf*, *pemel* etc. were down-regulated in WP and BP group compared with PB group.

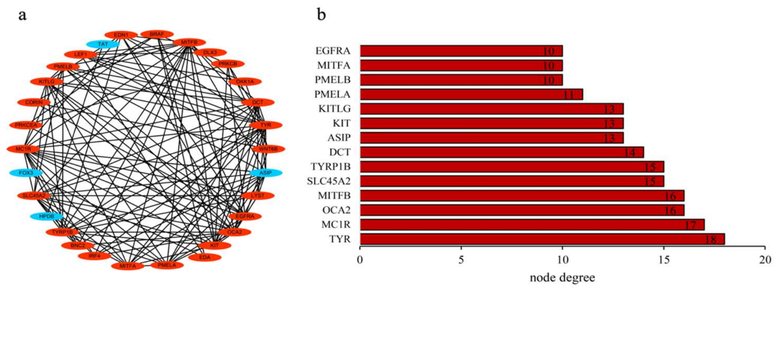

Fig. 5 The mutual protein-protein interactions of candidate genes in PB_WP comparison. a, PPI network; b, The genes of the PPI according to the node degree over 10

The genes (or proteins) from the pigment-related pathway were integrated together, and the relationship of them were extensive and strong (Fig. 5a). Among them, *asip*, *tat*, *hpdb* and *fox3* genes were significantly down-regulated, and other genes were significantly up-regulated. In addition, fourteen hub nodes in a PPI network with more than 10 nodes degree were shown in Fig. 5b.

Conclusion

- **KEGG analysis revealed numerous signaling pathways related to skin color regulation and pigmentation, such as oxidative phosphorylation, ribosome, Wnt signaling pathway, MAPK signaling pathway, melanogenesis, tyrosine metabolism *etc.***
- **A number color-related genes, including *tyrp1*, *tyr*, *pmel*, *mitf*, *mc1r*, *asip*, *tat*, *hpdb* and *foxd3*, *etc.* might play a potential role in skin color variation of red tilapia.**