Biochemical Genetics (2019) 57:734–744
https://doi.org/10.1007/s10528-019-09929-x

ORIGINAL ARTICLE

Morphological Characterization and Gene Expression Patterns for Melanin Pigmentation in Rex Rabbit

Shuaishuai Hu[1] · Pin Zhai[2] · Yang Chen[1] · Bohao Zhao[1] · Naisu Yang[1] · Manman Wang[1] · Yeyi Xiao[1] · Guolian Bao[3] · Xinsheng Wu[1]

Received: 25 July 2018 / Accepted: 27 June 2019 / Published online: 13 July 2019

Abstract

Animal melanin has an important role in the formation of animal fur and skin, which is determined by its quantities, character, and distribution. To identify the effect of melanin on the formation of multi-colored Rex rabbits (Black, Chinchilla, Beaver, Protein cyan, Protein yellow, White), the structure of hair follicles and melanin content in multi-colored Rex rabbit skins were observed by Hematoxylin and Eosin (H&E) staining and melanin staining, respectively. The melanin granules were primarily found in the epidermis and hair follicle roots. The melanin content of skin was measured by extracting melanin from skin tissue. The results demonstrated that the melanin content was the highest in the skin of black Rex rabbit. Additionally, we measured the mRNA and protein expression levels of melanin-related key genes (MITF and TYR) in the skin of different hair color by quantitative real-time PCR and Wes assay, respectively. The results revealed that the mRNA expression levels in the skin of black Rex rabbit was highly expressed when as compared with other Rex rabbit skin ($P < 0.01$), and they were the lowest in the skin of white Rex rabbit. Finally, correlation analysis was conducted between melanin content and the expression levels of mRNA and protein. The results indicated a significant correlation between melanin content and the mRNA expression of MITF ($P < 0.05$), but it was not correlated with the mRNA expression of TYR ($P > 0.05$). In summary, melanin deposition has important economic value, and the coat color of fur-bearing animals is partly determined by the melanin-related genes.

Keywords Rex rabbit · Skin · Melanin · Gene expression

✉ Xinsheng Wu
xswu@yzu.edu.cn

[1] College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, Jiangsu, China

[2] Animal Husbandry and Veterinary Research Institute, Jiangsu Academy of Agricultural Sciences, 210014 Nanjing, Jiangsu, China

[3] Animal Husbandry and Veterinary Research Institute, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, Zhejiang, China

Introduction

Fur color is an important economic trait for fur-bearing animals, and has important economic value. The coat color is determined by the quantities, character, distribution, and forms of melanin. Multi-colored Rex rabbits are valuable fur-bearing animals that serve as a good experimental model for studying animal melanin deposition. It is well known that fur color is extremely correlated with melanin deposition, and melanocytes are formed in the epidermis basal layer of the skin and in hair follicles (Riley 1997). Some studies have been found that melanocytes are detected in the early hair follicles of human (Tobin 1998). And it has been demonstrated that endocrine factors were very important effects on inducing changes of skin color during or aging (Costin and Hearing 2007). Melanin is the primary pigment in animal coloration produced by melanocytes, including pheomelanin and eumelanin, and some of its functions are caused by differential distributions and concentrations in mammals and birds. Previous research has reported that the coloration of different parts of feathers is determined by the proportion of pheomelanin and eumelanin in males and females (Saino et al. 2013). It has also been reported that epidermal melanin has an important role in evolutionary and physiological implications in humans (Slominski et al. 2004). In addition, the hair, skin, and eye color of animals can also be determined by the density and distribution of melanin (Zhu et al. 2010). However, melanin synthesis is very complex. It is determined by the migration of melanocytes, the synthesis of melanosome, and the transport of melanin granules, and it is regulated by several related genes.

Currently, there are approximately 150 genes that have been found to be involved in the development of fur color (Reissmann and Ludwig 2013), which can regulate the proportion of pheomelanin and eumelanin and the melanin deposition in the process of melanogenesis, further determine the production of different coat color. The MITF is a key downstream gene that regulates the melanogenesis signaling pathway, which regulates the expression of the tyrosine gene family to participate in the regulation of melanogenesis. Previous research has shown that MITF can determine the expression of MC1R gene to regulate coat color in cattle (Saber et al. 2014). And it has also been demonstrated that an obvious mutation in the MITF gene which associated with White phenotype was discovered in German Fleckvieh cattle (Philipp et al. 2011). Moreover, Changes in MITF function can directly lead to metabolic disorders of the pigment cell, and it plays an important role in the development and differentiation of melanocytes derived from the neural crest (Guibert et al. 2004; Kasamatsu et al. 2008; Philipp et al. 2011). In addition, tyrosine metabolism is regulated by TYR and TYRP1 of the Tyrosine gene family, and it directly regulates the production of melanin. Previous research has shown that MITF and TYR genes were significantly expressed in the sheep skin melanocytes (Pang et al. 2016). Therefore, the main goals for this research are to determine the effect of the melanin deposition on the formation of different coat color in Rex rabbits. In addition, the melanin-related genes play an important role in regulating the melanin deposition.

Materials and Methods

Ethics Statement

This study was carried out in accordance with the recommendations of Animal Care and Use Committee at Yangzhou University. The protocol was approved by the Animal Care and Use Committee at Yangzhou University. All animal experiments using anesthetized animals were conducted with regulation according to the Animal Management Rule of Yangzhou University (Jiangsu, China), and they were performed in a standard room with precise experimental conditions. Operational procedures were stringently conducted in accordance with Laboratory Animal Requirements of Environment and Housing Facilities (GB14925-2001). Overall, all work was done to reduce pain as much as possible.

Collection of Tissues

Black, Chinchilla, Beaver, Protein cyan, Protein yellow, and White Rex rabbits were provided by Zhejiang Yuyao Xinnong Rabbit Industry Co., Ltd., and three biological rabbits for each of coat color were kept under the same environmental conditions. At the 90th day of growth after birth, three biological samples of dorsal skin of the same size and position were collected to be used to make paraffin slices, determination of the melanin content, and total RNA extraction, respectively.

Paraffin Slicing

The skin samples were removed, and the shape was repaired. It was then fixed, dehydrated, cleared, and imbedded in wax. The wax blocks were sliced to produce paraffin sections of a thickness of 5 μm, and the sliced samples were heated in a water bath at 48 °C. Slices were picked up with a glass slide and baked at 60 °C for 12 h. Finally, the glass slides were stained, sealed, observed, and analyzed under a microscope.

Determination of Melanin Content of Skin Tissue

The skin samples were homogenized and added to PBS, papain, and trypsin. It was then heated in a water bath at 55 °C for 12 h, centrifuged at 8000 rpm for 10 min, and the supernatant was discarded. The sample was then washed by petroleum ether, anhydrous ethanol, and distilled water and freeze-dried. Finally, the samples were added to 0.1 mol/L NaOH and heated at 100 °C for 12 h. We formulated a melanin standard sample of 1 mg dissolved in 1 mol/L NaOH to give a final concentration of 100 μg/mL. It was heated in a water bath for 1.5 h at 55 °C. Then, to make a melanin standard curve, the following concentrations were made: 0, 10, 20, 30, 40, 50, 60, 70, and 80 μg/mL. For each concentration, 200 μL was added to each well, and then 200 μL of prepared skin samples was added to each well. Detection of the melanin standard sample at different concentrations and the prepared skin samples were determined by the Tecan Infinite® 200 Pro (Thermo Fisher Scientific) at an absorbance of A_{500}. The data were analyzed by IBM SPSS Statistics V22.0.0.

RNA Isolation and cDNA Preparation

Total RNA was isolated from skin tissue using a total RNA extraction kit (Tiangen Biotech (Beijing) Co., Ltd.), according to the instructions provided by the manufacturer. The single-stranded cDNA samples were prepared from 1 μL total RNA per reaction using the Super RT cDNA Kit (manufactured by Tiangen Biotech Co., Ltd.) and stored at − 20 °C for qRT-PCR assays.

Primer Design and Synthesis

To determine the expression levels of MITF and TYR in the skin of Rex rabbits, qRT-PCR assays were performed. Primers for MITF (NC_013677.1), TYR (NC_013669.1), and GAPDH (NC_013676.1) genes were designed according to the GenBank mRNA sequences using the Primer-BLAST online primer design program on the NCBI website. The primers were synthesized by the Shanghai Biological Engineering Co., Ltd., and primer sequences of these genes are listed in Table 1.

qRT-PCR Assay

The analysis of the qRT-PCR was performed using AceQ® qPCR SYBR® Green Master Mix purchased from Vazyme Biotech Co., Ltd, and the reaction system was optimized according to the manufacturer's instructions. Samples were prepared on ice, and each sample was set up in triplicate. The change in the fluorescence signal was analyzed by QuantStudio® 5 (Applied Biosystems, USA). The Ct value of each sample was obtained, and the GAPDH gene was used as an internal reference gene.

Table 1 RT-PCR primer sequences

Primer	Sequence (5′→3′)	Product length (bp)
MITF	Forward: GTGAGTCAGACACCAGCCATAA	142
	Reverse: CTTTACCTGCTGCCTTTGG	
TYR	Forward: GAGGGATGCAGAAAGCTG	134
	Reverse: TGTACTCCTCCAACCGGC	
GAPDH	Forward: CCAGGGCTGCTTTTAACTCT	141
	Reverse: TCCCGTTCTCAGCCTTGACC	

Isolation of Proteins and WES Assay

To determine the protein levels of MITF and TYR in the skin of multi-colored Rex rabbits, protein lysates from skin samples from six different coat colors were obtained using RIPA Lysis Buffer (PPLYGEN). Protein concentrations were determined using the Enhanced BCA Protein Kit (Beyotime). Protein lysates were diluted to 2 µg/µL using 0.1 × sample buffer, and 7.5 µg protein was detected. The results were analyzed with the Wes automated Western blot system purchased from Protein Simple (Harris 2015). The following antibodies were used: anti-GAPDH mouse monoclonal antibody diluted at 1:100 (Abcam), anti-MITF antibody produced in rabbit diluted at 1:100 (Sigma-Aldrich), and anti-TYR mouse monoclonal antibody diluted at 1:100 (BBI). The protein levels of different samples were analyzed by quantifying the gray value and calculating the area under the curve.

Statistical Analysis

The relative quantitative results were analyzed using the $2^{-\Delta\Delta Ct}$ method. The correlation analysis between the melanin content and the expression level of mRNA and protein was performed by Pearson Correlation in IBM SPSS Statistics V22.0.0. Significant differences among the different coat colors were analyzed using one-way ANOVA with Duncans multiple comparison test in IBM SPSS Statistics V22.0.0, and all data had been tested using homoscedasticity in one-way ANOVA. All data are presented as mean ± standard error.

Results

Distribution of Hair Follicles in the Dermis Layer of Rex Rabbits

The paraffin slices of skin tissues were stained with melanin staining. It was shown that the epidermis and dermis of the skin were markedly differentiated. The distribution of hair follicles was virtually the same among the different skin samples, and they were distributed in the dermis layer. Melanin granules were observed in the hair follicles (Fig. 1).

The samples of the skin tissues were examined by H&E staining. We observed that the primary follicles and secondary follicles existed in the skin samples, and every follicle group consisted of primary and secondary follicles. The primary follicles were formed during the early stage of hair follicle development, and the glands were abundant in the primary follicles. In addition, there were also subsidiary structures, including sweat glands, sebaceous glands, and arrector pili muscles. However, there were few sebaceous glands in the secondary follicles found in late stages. As shown in Fig. 2, the melanin granules were found in hair follicles.

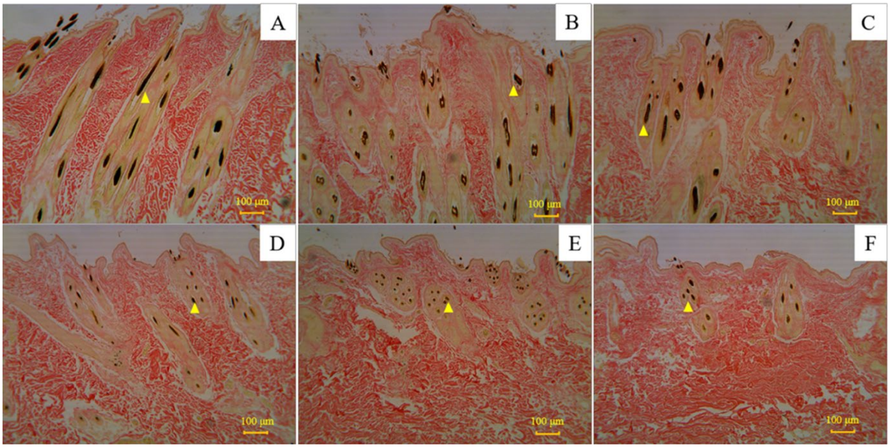

Fig. 1 The distribution of hair follicles and melanin granules in the skin (100×). The paraffin slices of skin tissues were examined with melanin staining, and this figure showed was longitudinal section. **a** Black, **b** Chinchilla, **c** Beaver, **d** Protein cyan, **e** Protein yellow, **f** White, △ melanin granules

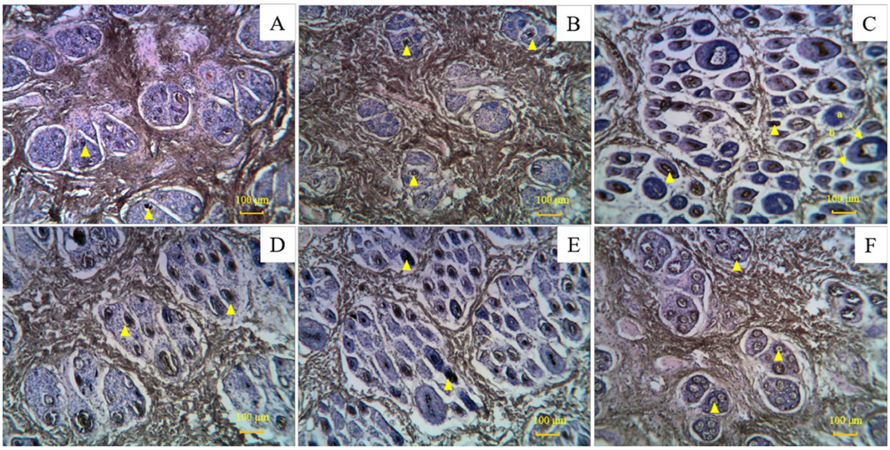

Fig. 2 Hair follicle structure in the skin of Rex rabbits (100×) The skin tissue samples were stained by H&E staining, and this figure displayed was transverse section. **a**: Black, **b** Chinchilla, **c** Beaver, **d** Protein cyan, **e** Protein yellow, **f** White, △ melanin granules

Detection of Melanin in the Skin of Rex Rabbits

The concentration of melanin was detected by the Tecan Infinite® 200 Pro at an absorbance of A_{500}. As shown in Table 2, the melanin content was significantly different among the skin of different coat colors. The melanin contents of black Rex rabbit's skin were the highest. They were significantly higher than the skin of beaver, protein cyan, protein yellow, and white Rex rabbits ($P < 0.05$), but it was not significantly different with the Chinchilla. The melanin content was the lowest in the white Rex rabbit's skin.

Table 2 The melanin content in the skin of different coat colors

	Coat color					
	BL	CH	BR	PC	PY	WH
Absorbance value (A500)	3.068 ± 0.027^{a}	2.985 ± 0.258^{a}	2.683 ± 0.155^{b}	2.591 ± 0.071^{b}	2.551 ± 0.115^{b}	2.253 ± 0.142^{c}
Concentration of melanin (μg/mL)	57.458 ± 0.508^{a}	55.877 ± 4.885^{a}	50.141 ± 2.936^{b}	48.398 ± 1.341^{b}	47.632 ± 2.184^{b}	41.981 ± 2.702^{c}

The melanin content was determined at an absorbance of A500, and concentration of melanin was calculated according to the absorbance of A500. And significant differences among the different coat colors were analyzed using one-way ANOVA with Duncans multiple comparison test in IBM SPSS Statistics V22.0.0. In the same row, values with different small letter superscripts signify a significant difference ($P < 0.05$)

BL Black, *CH* Chinchilla, *BR* Beaver, *PC* Protein cyan, *PY* Protein yellow, *WH* White

mRNA Expression of MITF and TYR Genes in the Skin of Rex Rabbits

As shown in Fig. 3, the mRNA expression levels of MITF and TYR in the skin of different coat colors were significantly different. The expression levels of these genes in black Rex rabbit's skin were significantly higher than in other colors, and the lowest expression was found in the white Rex rabbit's skin. Furthermore, the mRNA expression in the Protein yellow and white Rex rabbit's skins was not significantly different for MITF, but there was a significant difference among the other coat colors ($P < 0.05$). The mRNA expression levels of TYR in black Rex rabbit's skin was highly expressed ($P < 0.01$), but it was rarely expressed in the white Rex rabbit's skin.

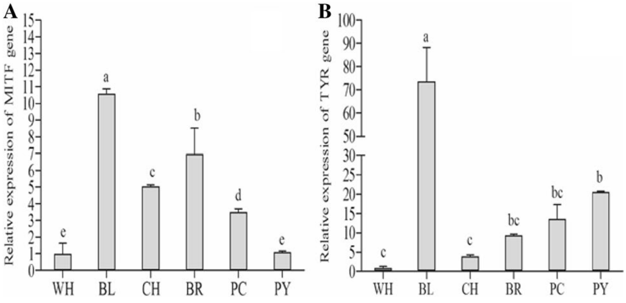

Fig. 3 The mRNA expression levels of different genes in the different skin colors. The expression levels of MITF and TYR in the skin of Rex rabbits were determined by Real-time PCR assays, and the relative expression were analyzed using the $2^{-\Delta\Delta Ct}$ method. Significant differences among the groups indicated by a–d ($P < 0.05$). BL: Black, CH: Chinchilla, BR: Beaver, PC: Protein cyan, PY: Protein yellow, WH: White

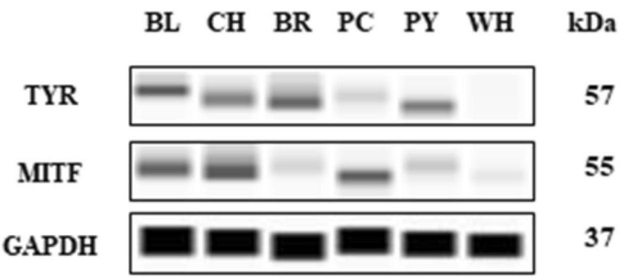

Fig. 4 The protein levels of MITF and TYR in the different skin colors. The protein expression levels of MITF and TYR in different skin colors were determined by the Wes automated Western blot system. Protein lysates were diluted to 2 µg/µL using 0.1 × sample buffer, and 7.5 µg protein was detected in each well. BL: Black, CH: Chinchilla, BR: Beaver, PC: Protein cyan, PY: Protein yellow, WH: White

Table 3 Quantification of different samples using the area under the curve

Protein	BL	CH	BR	PC	PY	WH
TYR	3286	3270	3281	1059	2947	740
MITF	4489	2976	1362	1705	938	325
GAPDH	15,960,334	13,838,052	13,973,444	14,094,027	13,803,513	11,503,765

The protein expression levels of MITF and TYR in different skin colors were determined by Western blot, and analyzed by quantifying the gray value and calculating the area under the curve

BL Black, *CH* Chinchilla, *BR* Beaver, *PC* Protein cyan, *PY* Protein yellow, *WH* White

Table 4 Correlation between gene expression and concentration of melanin in different skin colors

Gene expression	MITF	TYR
Concentration of melanin (μg/mL)	0.822*	0.604

Correlation analysis between concentration of melanin and mRNA expression levels of MITF and TYR was performed by Pearson correlation in IBM SPSS Statistics V22.0.0

Significant difference denoted by * ($P<0.05$); highly significant difference denoted by ** ($P<0.01$)

Protein Expression Levels of Key Genes in the Skin of Rex Rabbits

The protein expression levels of MITF and TYR in the skin of different coat colors were determined by Wes analysis. As shown in Fig. 4, these genes were all expressed in the different coat colors. The protein expression was different for these genes, and the protein expression of the skin of light coat color was significantly lower than in the skin of dark coat color ($P<0.01$). This could be due to a lack of melanin in the light coat color. The quantitation of protein levels in different coat color's skins was performed utilizing the area by Wes capillary western blotting. As shown in Table 3, the protein level of MITF and TYR genes in black Rex rabbit's skin was significantly higher than the other coat colors ($P<0.01$), and the lowest expression levels were found in white Rex rabbit's skin.

Table 5 Correlation between protein expression and concentration of melanin in different skin colors

Protein expression	MITF	TYR
Concentration of melanin (μg/mL)	0.939**	0.761

Correlation analysis between concentration of melanin and protein expression levels of MITF and TYR were performed by Pearson Correlation in IBM SPSS Statistics V22.0.0

Significant difference denoted by *($P < 0.05$); highly significant difference denoted by **($P < 0.01$)

Correlation Between Melanin Content and Expression Levels of mRNA and Protein

Correlation analysis was performed by Pearson Correlation in SPSS v. 22.0 software. As shown in Table 4, the results clearly demonstrate a significant positive correlation between the melanin content and the mRNA expression of MITF ($P < 0.05$), but it was not correlated with the mRNA expression of TYR ($P > 0.05$). As shown in Table 5, there was a highly significant correlation between melanin content and protein expression of MITF ($P < 0.01$).

Discussion

Hair follicles in the skin have an important role in inducing hair regrowth, and the decrease of hair indicates aging in the many mammals (Matsumura et al. 2016). It has also been found that Wnt3a, an important Wnt family member, can promote melanin synthesis in hair follicle melanocytes in mice (Guo et al. 2012). Melanin has not only an impact on animal coloration, but also can regulate high stiffness of Retinal pigment epithelium cells, and support mechanical completeness of the blood-retinal barrier tissue (Sarna et al. 2017). In addition, Previous research has shown that severity of epithelial dysplasia was correlated with the decrease in amount of melanin granules and melanocyte (Honwad et al. 2017). In this study, melanin granules and the structure of hair follicles were clearly observed in the skins by melanin staining and H&E staining, and it was obviously examined in the structure of cells (Na et al. 2006). The structure of melanin granules in different species is also diverse. Some studies have shown that the magpie feathers have a complex structure of melanin granules, but the structure of melanin granules of the jungle crow is simple (Lee et al. 2010). Therefore, the structure of the melanin granules in Rex rabbits is different from other species. Different colors are associated with different melanin granules, and some research has suggested that the amount of melanin granules in the white hairs are rather few (Lee and Chang 2013). This is consistent with our results shown in Fig. 2 that exhibit fewer melanin particles in the skin of white Rex rabbits than in the skin of Beaver and Protein yellow Rex rabbits. The mRNA expression levels of MITF and TYR genes in the skin of white Rex rabbits were significantly lower than that of the other coat colors ($P < 0.01$). MITF is an important transcription factor that controls the genes associated with pigmentation (Bemis et al. 2008), and it plays an essential role in melanocyte development and differentiation (Yajima et al. 2011). Studies have found that the relative expression of the MITF gene in the skin of brown alpaca is significantly higher than the skin of white alpaca (Zhu et al. 2010). Therefore, it is speculated that the expression of MITF in the skin of various colored Rex rabbits differs. Our results support this hypothesis. The mRNA and protein expression of the MITF gene is different among the skin of multi-colored Rex rabbits, and the mRNA and protein expression of MITF are the lowest in the skin of white Rex rabbit. In addition, the concentration of melanin in the skins of different coat colors had a highly significant correlation with the protein expression of MITF ($P < 0.01$). Notably, the TYR gene is a fairly critical gene for melanogenesis and deposition. We found that TYR expression was the highest in the Black Rex rabbit's skin, but its mRNA and protein expression was the lowest in the skin of white Rex rabbits. However, the synthesis of melanin is associated with the expression of TYR mRNA and protein, and it is regulated at the post-translational level (Watabe et al. 2004). The formation of pheomelanin and eumelanin contributes significantly to its expression. And previous research has also demonstrated that the expression levels of TYR, TYRP1, and DCT determined the content of eumelanin, but not correlated with pheomelanin (Guibert et al. 2004). Therefore, eumelanin synthesis activates TYR, and the production of pheomelanin inactivates TYR. Taken together, our results indicate that the amount of melanin is the highest in the skin of Black Rex rabbits, and MITF and TYR genes play an important role in the process of promoting melanin synthesis.

Conclusions

Generally, melanin plays an important role in process of melanogenesis, and melanin deposition has an important economic value. In this study, the melanin granules were found in the epidermis and hair follicle roots, and the melanin content was the highest in the black Rex rabbit's skin. In addition, the mRNA expression levels in the black Rex rabbit's skin was also highly expressed when as compared with other coat colors ($P < 0.01$), but they were the lowest in the white Rex rabbit's skin. Moreover, there was a significant correlation between melanin content and the mRNA expression of MITF ($P < 0.05$), but it was not significantly correlated with the mRNA expression of TYR ($P > 0.05$). In summary, the coat color of the fur-bearing animal is greatly interrelated with the expression levels of melanin-related genes, so our findings further support the theory of melanin deposition of fur-bearing animal.

Funding This work was supported by the Modern Agricultural Industrial System Special Funding (CARS-43-A-1), the National Natural Science Foundation of China (3170130120), and the Science and Technology Major Project of New Variety Breeding (Livestock and Poultry) of Zhejiang Province, China (2016C02054-10).

Compliance with Ethical Standards

Conflict of interest ShuaiShuai Hu, Yang Chen, BoHao Zhao, NaiSu Yang, ManMan Wang, Yeyi Xiao, Guolian Bao, and Xinsheng Wu declare that they have no conflict of interest.

References

Bemis LT, Chen R, Amato CM, Classen EH, Robinson SE, Coffey DG (2008) MicroRNA-137 targets microphthalmia-associated transcription factor in melanoma cell lines. Can Res 68:1362–1368

Costin GE, Hearing VJ (2007) Human skin pigmentation: melanocytes modulate skin color in response to stress. Faseb J 21:976–994

Guibert S, Girardot M, Leveziel H, Julien R, Oulmouden A (2004) Pheomelanin coat colour dilution in French cattle breeds is not correlated with the TYR, TYRP1 and DCT transcription levels. Pigment Cell Res 17:337–345

Guo H, Yang K, Deng F, Ye J, Xing Y, Li Y (2012) Wnt3a promotes melanin synthesis of mouse hair follicle melanocytes. Biochem Biophys Res Commun 420:799–804

Harris VM (2015) Protein detection by Simple Western™ analysis. Methods Mol Biol 1312:465–468

Honwad S, Ravi SV, Donoghue M, Joshi M (2017) Immuno-histochemical and quantitative study of melanocytes and melanin granules in oral epithelial dysplasia. J Clin Diagn Res 11:ZC56–ZC58

Kasamatsu S, Hachiya A, Higuchi K, Ohuchi A, Kitahara T, Boissy RE (2008) Production of the soluble form of KIT, s-KIT, abolishes stem cell factor-induced melanogenesis in human melanocytes. J Investig Dermatol 128:1763–1772

Lee JY, Chang BS (2013) Morphogenetic types of cuticles and melanin granules in the anagen phase of hair follicles. Sci Res Essays 8:807–814

Lee E, Lee H, Kimura J, Sugita S (2010) Feather microstructure of the black-billed magpie (*Pica pica sericea*) and jungle crow (*Corvus macrorhynchos*). J Vet Med Sci 72:1047–1050

Matsumura H, Mohri Y, Binh NT, Morinaga H, Fukuda M, Ito M (2016) Hair follicle aging is driven by transepidermal elimination of stem cells via COL17A1 proteolysis. Science 351:aad4395

Na GY, Paek SH, Park BC, Kim DW, Kim JC (2006) Isolation and characterization of outer root sheath melanocytes of human hair follicles. Br J Dermatol 155:902–909

Pang Y, Geng J, Qin Y, Wang H, Fan R, Zhang Y (2016) Endothelin-1 increases melanin synthesis in an established sheep skin melanocyte culture. Vitro Cell Dev Biol 52:749–756

Philipp U, Lupp B, Stefanie M, Stein V, Tipold A, Eule JC (2011) A MITF mutation associated with a dominant white phenotype and bilateral deafness in german fleckvieh cattle. PLoS ONE 6:e28857

Reissmann M, Ludwig A (2013) Pleiotropic effects of coat colour-associated mutations in humans, mice and other mammals. Semin Cell Dev Biol 24:576–586

Riley PA (1997) Melanin. Int J Biochem Cell Biol 29:1235–1239

Saber Q, Hubert P, Sandra J, Mehmet S, Tim MS, Ruedi F, Rasmus N, Henner S (2014) Classic selective sweeps revealed by massive sequencing in cattle. PLoS Genet 10:e1004148

Saino N, Maria R, Diego R, Celine T, Roberto A, Manuela C, Luca C, Kazumasa W (2013) Sexual dimorphism in melanin pigmentation, feather coloration and its heritability in the barn swallow (*Hirundo rustica*). PLoS ONE 8:e58024

Sarna M, Olchawa M, Zadlo A, Wnuk D, Sarna T (2017) The nanomechanical role of melanin granules in the retinal pigment epithelium. Nanomed Nanotechnol Biol Med 13:801–807

Slominski A, Tobin DJ, Shibahara S, Wortsman J (2004) Melanin pigmentation in mammalian skin and its hormonal regulation. Physiol Rev 84:1155–1228

Tobin DJ (1998) A possible role for Langerhans cells in the removal of melanin from early catagen hair follicles. Br J Dermatol 138:795–798

Watabe H, Valencia JC, Yasumoto KI, Kushimoto TA, Muller J (2004) Regulation of tyrosinase processing and trafficking by organellar pH and by proteasome activity. J Biol Chem 279:7971–7981

Yajima I, Kumasaka MY, Thang ND, Goto Y, Takeda K, Iida M (2011) Molecular network associated with MITF in skin melanoma development and progression. J Skin Cancer 2011:1–7

Zhu Z, He J, Jia X, Jiang J, Bai R, Yu X, You R (2010) MicroRNA-25 functions in regulation of pigmentation by targeting the transcription factor MITF in alpaca (*Lama pacos*) skin melanocytes. Domest Anim Endocrinol 38:200–209