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SHORT COMMUNICATION

Downregulation of c-Kit/MITF-M in Graying Hair of Juvenile Poliosis

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Juvenile poliosis, also known as premature canities, occurs in young adults. The incidence of this condition is about 15–32% in the Chinese population. The mechanism of juvenile poliosis is currently poorly understood but is thought to be related to inheritance and nutrition.

Hair pigmentation depends entirely on the presence of melanin, produced by melanocytes. The active melanocytes of the bulb are essential, because they produce and transfer melanin to the cortical keratinocytes of the shaft (1, 2). A reduction in tyrosinase (TYR) activity, suboptimal melanocyte-cortical keratinocyte interactions, and defective migration of melanocytes can all lead to pigment dilution or true grayness (3).

Melanocytes of the follicular pigmentary unit are derived from the neural crest and melanogenesis is tightly regulated by several factors, including microphthalmia-associated transcription factor (MITF), the Paired box 3 (Pax3), the Sry-related HMG box 10 (Sox10), tyrosine kinase receptor c-Kit and others (4). MITF-M isoforms have been reported to be expressed in the hair bulb and in the melanocyte stem cells in the bulge area. MITF-M is a key transcription factor regulating the expression of tyrosinase, and is also associated with the migration, survival, and differentiation/proliferation of melanocytes (5). Pax3 can increase MITF expression by binding to its promoter and simultaneously prevent MITF from activating downstream genes by competition for enhancer occupancy in melanocyte precursors. Sox10 is expressed in melanoblasts, which can directly transactivate the MITF gene and cooperate with MITF to activate tyrosinase expression (6, 7). The tyrosine kinase receptor, c-Kit, is also important for melanocyte survival during development, and mutations in these genes result in unpigmented hairs (8). Furthermore, immature melanocytes must express c-Kit as a prerequisite for migration into the stem cell factor-supplying hair follicle epithelium. Differentiating c-Kit-positive melanocytes appear to target the hair bulb (4).

The present study seeks to explore the expression of genes related to melanogenesis in relation to the mechanism of juvenile poliosis.

MATERIALS AND METHODS

Thirty-five participants with juvenile poliosis were studied: 21 patients (13 males, 8 females; age range 16–25). Hair samples from 14 healthy controls (9 males, 5 females; age range 18–25) were also examined. Subjects with vitiligo, other diseases of the hair, or systemic diseases were excluded. Both white and black hair samples were plucked, along with bulbs. For each patient 60 samples of white hair were taken.

RNeasy FFPE Kit was obtained from Qiagen Corporation; RevertAid First Strand cDNA Synthesis Kit was supplied from Thermo Fisher Scientific Incorporation. PCR mix (2 × Power Taq PCR MasterMix) was provided by Bioteke Biotechnology. All the primers were ordered from AuGCT Biotechnology.

Total RNA was extracted from white and black hair bulbs according to the RNeasy FFPE Kit protocol. Approximately 3 µg of total RNA was used to generate 20 µl cDNA. After reverse transcription with oligo$(dT)_{18}$ primers, semi-quantitative PCR was performed with 2 µl of cDNA to determine the levels of mRNA. Primers for each gene are listed in Table SI[1]. The following conditions were used in the PCR reaction to amplify MITF-M, TYR and c-Kit transcripts: 95°C for 3 min, 40 cycles of 95°C for 30 s, 58°C for 30s, 72°C for 30 s, followed by extension at 72°C for 8 min. Pax3 and Sox10 were amplified by following conditions: 95°C for 3 min, 35 cycles of 95°C for 30 s, Pax3 –58°C and Sox10 –62°C for 30s, 72°C for 20 s, followed by extension at 72°C for 8 min. After PCR amplification, 5 µl of the products were visualised by gel electrophoresis (2% agarose gel containing ethidium bromide). Band intensities were monitored by densitometric scanning, standardised against GAPDH as a reference gene from parallel reactions.

Results shown are representative of the whole experiment performed in triplicate and are presented as the means ± standard deviation (SD). The statistically significant differences were evaluated by independent sample *t*-tests, and *p*-values of < 0.05 were considered statistically significant.

RESULTS

The level of TYR mRNA expression decreased in juvenile poliosis hair samples compared with black hair ($p = 0.022$). The expression of MITF-M in white hair bulbs with juvenile poliosis was significantly lower than that seen in those from black hairs ($p = 0.001$). There was no significant difference in Pax3 or Sox10 mRNA expression between juvenile poliosis and control samples ($p = 0.493$, $p = 0.844$). On the other hand, c-Kit was expressed weakly in white hair compared to black hair ($p = 0.003$) (Table I, Fig.1).

DISCUSSION

We report that the expression of some key factors of melanogenesis (TYR, MITF-M, and c-Kit) is reduced in juvenile poliosis hair samples, whereas Pax3 and Sox10

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Table I. *Analysis of the expression of TYR, MITF-M, Pax3, Sox10 and c-Kit in juvenile poliosis and normal controls*

Gene	Relative optical density[a] Black hair Mean ± SD	White hair Mean ± SD	*t*-values	*p*-values
TYR	12.63 ± 7.21	6.18 ± 3.47	2.52	0.022
MITF-M	17.77 ± 10.48	3.39 ± 1.80	4.68	0.001
Pax3	12.78 ± 10.27	10.17 ± 8.21	0.69	0.493
Sox10	5.15 ± 2.89	5.41 ± 3.74	0.20	0.844
c-Kit	11.67 ± 3.76	5.56 ± 2.27	3.60	0.003

[a]The ratio of gene/GAPDH was calculated to obtain relative optical density.

expression showed no obvious changes compared with normal black hair.

TYR, an enzyme uniquely expressed in melanocytes, catalyses the rate-limiting initial stages of melanogenesis. The constitutive colour of an individual's hair is strongly influenced by tyrosinase activity (4, 9). Our findings indicate that mRNA expression of TYR declined in juvenile poliosis. Choi et al. (10) demonstrated that the expression of MITF-M, Pax3 and Sox10 is absent in the bulbs of white hair in the elderly, and concluded that hair greying is caused by defective migration of melanocyte stem cells into the bulb area of hair. In our experiment, only the expression of MITF-M decreased, while Pax3 and Sox10 did not change significantly in the white hair bulbs of juvenile poliosis. This suggests that premature canities differ from hair greying seen in the older population. As melanocyte stem cells or melanocytes express markers for neural crest cells, Pax3 and Sox10, our results suggest that the number of melanocytes or melanocyte stem cells is not significantly reduced, and the hair greying could be attributed to the dysfunction of melanocytes. Thus, we speculate that it is the lowered expression of MITF-M in melanocytes which caused TYR-reduced expression in the graying hair follicles.

MITF-M is the downstream of stem cell factor/c-Kit signalling. The stem cell factor activation of c-Kit receptor leads to MITF phosphorylation through the MAP kinase pathway (11, 12). Thus, the weak expression of c-Kit in our experiment could lead to decreased expression of MITF-M. Botchkareva et al. (8) demonstrated that anti-c-Kit antibody could dose-dependently decrease hair pigmentation and lead to partially or fully depigmented hairs. Additionally, melanocyte stem cells are not dependent on stem cell factor/c-Kit and when appropriately stimulated can generate melanogenically active melanocytes. As it is not too uncommon to see spontaneous repigmentation along the same individual hair shaft in early canities, the results provide evidence that the inhibition of c-Kit signalling is one of the key pathways for hair depigmentation in juvenile poliosis.

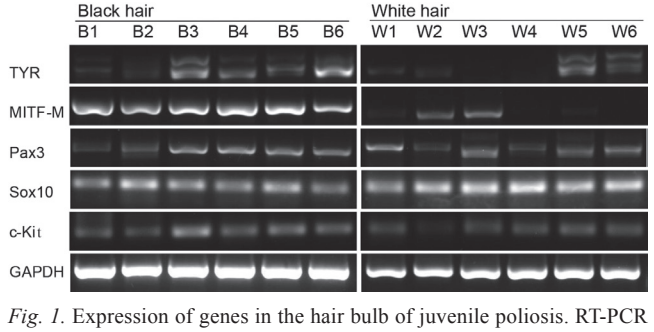

Fig. 1. Expression of genes in the hair bulb of juvenile poliosis. RT-PCR analysis was performed for TYR, MITF-M, Pax3, Sox10, c-Kit and GAPDH. The representative lanes of juvenile poliosis (White hair) and healthy control (Black hair) are shown. GAPDH was used as an internal control.

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REFERENCES

1. Anbar TS, Abdel-Raouf H, Awad SS, Ragaie MH, Abdel-Rahman AT. The hair follicle melanocytes in vitiligo in relation to disease duration. J Eur Acad Dermatol Venereol 2009; 23: 934–939.
2. Steingrímsson E, Copeland NG, Jenkins NA. Melanocyte stem cell maintenance and hair graying. Cell 2005; 121: 9–12.
3. Tobin DJ, Paus R. Graying: gerontobiology of the hair follicle pigmentary unit. Exp Gerontol 2001; 36: 29–54.
4. Tobin DJ. Human hair pigmentation – biological aspects. Int J Cosmet Sci 2008; 30: 233–257.
5. Wan P, Hu Y, He L. Regulation of melanocyte pivotal transcription factor MITF by some other transcription factors. Mol Cell Biochem 2011; 354: 241–246.
6. Harris ML, Baxter LL, Loftus SK, Pavan WJ. Sox proteins in melanocyte development and melanoma. Pigment Cell Melanoma Res 2010; 23: 496–513.
7. Kubic JD, Young KP, Plummer RS, Ludvik AE, Lang D. Pigmentation PAX-ways: the role of Pax3 in melanogenesis, melanocyte stem cell maintenance, and disease. Pigment Cell Melanoma Res 2008; 21: 627–645.
8. Botchkareva NV, Khlgatian M, Longley BJ, Botchkarev VA, Gilchrest BA. SCF/c-kit signaling is required for cyclic regeneration of the hair pigmentation unit. FASEB J 2001; 15: 645–658.
9. Slominski A, Wortsman J, Plonka PM, Schallreuter KU, Paus R, Tobin DJ. Hair follicle pigmentation. J Invest Dermatol 2005; 124: 13–21.
10. Choi YJ, Yoon TJ, Lee YH. Changing expression of the genes related to human hair graying. Eur J Dermatol 2008; 18: 397–399.
11. Kitamura R, Tsukamoto K, Harada K, Shimizu A, Shimada S, Kobayashi T, et al. Mechanisms underlying the dysfunction of melanocytes in vitiligo epidermis: role of SCF /KIT protein interactions and the downstream effector, MITF-M. J Pathol 2004; 202: 463–475.
12. Bear J, Hong Y, Schartl M. MITF expression is sufficient to direct differentiation of medaka blastula derived stem cells to melanocytes. Development 2003; 130: 6545–6553.