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L-tyrosine induces melanocyte differentiation in novel pink-eyed dilution castaneus mouse mutant showing age-related pigmentation

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ABSTRACT

Background: The mouse pink-eyed dilution (oculocutaneous albinism II; *p/Oca2*^{p}) locus is known to control tyrosinase activity, melanin content, and melanosome development in melanocytes. Pink-eyed dilution castaneus (*p*^{cas}/*Oca2*$^{p-cas}$) is a novel mutant allele on mouse chromosome 7 that arose spontaneously in Indonesian wild mice, *Mus musculus castaneus*. Mice homozygous for *Oca2*$^{p-cas}$ usually exhibit pink eyes and beige-colored coat on nonagouti C57BL/6 (B6) background. Recently, a novel spontaneous mutation occurred in the progeny between this mutant and B6 mice. The eyes of this novel mutant progressively become black from pink and the coat becomes dark gray from beige with aging.
Objective: The aim of this study is to clarify whatever differences exist in melanocyte proliferation and differentiation between the ordinary (pink-eyed) and novel (black-eyed) mutant using serum-free primary culture system.
Methods: The characteristics of melanocyte proliferation and differentiation were investigated by serum-free primary culture system using melanocyte-proliferation medium (MDMD).
Results: The proliferation of melanoblasts in MDMD did not differ between the two mice. However, when the epidermal cell suspensions were cultured with MDMD supplemented with L-tyrosine (Tyr), the differentiation of black-eyed melanocytes was greatly induced in a concentration-dependent manner compared with pink-eyed melanocytes. Immunocytochemistry demonstrated that the expression of tyrosinase and tyrosinase-related protein-1 (Tyrp1) was greatly induced or stimulated both in pink-eyed and black-eyed melanocytes, whereas the expression of microphthalmia-associated transcription factor (Mitf) was stimulated only in black-eyed melanocytes.
Conclusion: These results suggest that the age-related coat darkening in black-eyed mutant may be caused by the increased ability of melanocyte differentiation dependent on L-Tyr through the upregulation of tyrosinase, Tyrp1, and Mitf. This mutant mouse may be useful for animal model to clarify the mechanisms of age-related pigmentation in human skin, such as melasma and solar lentigines.

1. Introduction

The proliferation and differentiation of mammalian melanocytes are regulated by genetic and epigenetic factors. Of epigenetic factors, tissue environment surrounding melanocytes such as keratinocytes and fibroblasts is most important. On the other hand, coat color genes are the most important genetic factors that regulate melanocyte proliferation and differentiation during skin development [1–4]. More than 80 alleles have so far been reported [5]. The mouse pink-eyed dilution (*p/Oca2*^{p}, oculocutaneous albinism II) locus is one of the classical coat color loci and the locus controls tyrosinase activity, melanin synthesis, and melanosome development [6–11].

In humans, OCA2 is one of the albinisms and caused by the mutation in the human OCA2 that is orthologous to the mouse *Oca2* gene [12,13]. The inactivation of the human *OCA2* gene (chromosome 15q11-q13) results in Prader–Willi and Angelman syndromes [14].

The product of the *P* (*Oca2*) gene is an integral membrane protein that localizes in melanosomes [15]; its predicted secondary structure is a 12-transmembrane domain protein, which is similar to a channel or transporter [12,14]. The Oca2 protein seems to control the processing and transport of tyrosinase [11]. The Oca2 protein may not be a tyrosine transporter [16]. It is also reported that the pH of melanosomes is abnormal in *Oca2*^{p}/*Oca2*^{p} melanocytes [17]. Moreover, Sitaram et al. [18] reported that

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OCA2 protein was active in melanosomes and its activity might be limited by additional sorting to lysosomes.

The number of epidermal melanoblasts and melanocytes was greatly reduced in the neonatal skin of *Oca2*^{p}/*Oca2*^{p} mice [9]. In serum-free primary culture system, the proliferation and differentiation of neonatal mouse epidermal melanocytes were greatly inhibited by the *Oca2*^{p} mutation [9] and excess L-tyrosine (Tyr) supplemented to the culture medium rescued both proliferation and differentiation of *Oca2*^{p}/*Oca2*^{p} melanocytes [10]. In the culture system, most of melanins as well as their precursors failed to be accumulated in *Oca2*^{p}/*Oca2*^{p} melanocytes and were released from them [19,20]. Although *Oca2*^{p}/*Oca2*^{p} melanoblasts possessed only a few stage I and II melanosomes, L-Tyr rescued the formation of stage III and IV melanosomes in addition to stage I and II melanosomes [10,21]. Moreover, *Oca2*^{p}/*Oca2*^{p} mutation is involved in regulating the function of mitochondria [21], and the action of the *Oca2*^{p} allele on the proliferation and differentiation of melanocytes may be related to the apoptotic cell death [22]. Thus, the action of the *Oca2*^{p} allele affects diverse function of melanocytes through complicated pathway.

In 1990, a spontaneous mutation (Fig. 1A) of pink eyes and beige-colored coat occurred in Indonesian wild mice (*Mus musculus castaneus*) [23]. This phenotype is similar to that of classical *Oca2*^{p}/*Oca2*^{p} mice. Genetic study revealed that this mutant was allelic to *Oca2*^{p}/*Oca2*^{p}, and this new mutant allele was named pink-eyed castaneus (*p*^{cas}/*Oca2*$^{p-cas}$). Moreover, Ishikawa et al. [24] discovered a novel mutant of age-related darkening phenotype of eyes and coats in the offspring of F_3 generations between this mutant and C57BL/6JJcl (B6) mice. After 3 months, this novel mutant developed black eyes and darker coat (Fig. 1B) [24]. The pigmentation in choroid and hair follicles of this mutant was greatly increased compared with that of pink-eyed mice [24]. Molecular analysis demonstrated that *Oca2*$^{p-cas}$ possesses 4.1-kb deletion in exons 15 and 16. No sequence difference was observed between the two types of mutant. Genetic study revealed that this novel phenotype was regulated by two autosomal recessive genes, namely the *Oca2*$^{p-cas}$ and unknown modifier genes. However, this mutation exhibits incomplete penetrance and suggests the presence of epigenetic control [24].

In this study, to clarify the mechanisms of age-related pigmentation in coat of black-eyed mice, the characteristics of the proliferation and differentiation of melanoblasts/melanocytes were investigated in detail between pink-eyed and black-eyed mice using serum-free primary culture system.

2. Materials and methods

2.1. Mice

All animal works were carried out in accordance with the guidelines for the care and use of laboratory animals of Graduate School of Bioagricultural Sciences, Nagoya University, Japan. Ordinary (pink-eyed) and novel (black-eyed) mutant mice (*Mus musculus*) were maintained as B6;Cg-*Oca2*$^{p-cas}$/1Nga and B6;Cg-*Oca2*$^{p-cas}$/2Nga sub-mixed strains, respectively, both carrying the same *Oca2*$^{p-cas}$ gene derived from wild *M. m. castaneus* on the B6 genetic background at the Laboratory of Animal Genetics of Nagoya University [24]. All mice were reared in an environment with a temperature of 23 ± 2 °C and a light/dark cycle of 12:12 h. Commercial diet (CA-1, Clea, Tokyo, Japan) and water were provided *ad libitum*.

2.2. Melanocyte primary culture

The sources of tissue for the culture of melanoblasts and melanocytes were dorsal skins of pink-eyed and black-eyed mutant mice of ages from 0.5 to 2.5 days. Unless stated otherwise, all reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The method for obtaining epidermal cell suspensions was reported previously [9]. Briefly, disaggregated epidermal cell suspensions were pelleted by centrifugation and suspended in Ham's F-10 medium (Gibco, Grand Island, NY, USA). The cell pellet after centrifugation was resuspended in melanocyte-proliferation medium (MDMD) consisting of Ham's F-10 plus 10 μg/ml bovine insulin, 0.5 mg/ml bovine serum albumin (Fraction V), 1 μM ethanolamine, 1 μM phosphoethanolamine, 10 nM sodium selenite, 0.5 mM dibutyryl adenosine 3′:5′-cyclic monophosphate, 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate, 50 μg/ml gentamycin sulfate, and 0.25 μg/ml amphotericin B. The same lots of these supplements were used in this study. Cells in each epidermal cell suspension were counted in a hemocytometer chamber and plated onto type I collagen (Becton Dickinson, Bedford, MA, USA)-coated dishes [9] at an initial density of 1×10^6 cells/35 mm dish (1.04×10^5 cells/cm^2). Cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air (pH 7.2).

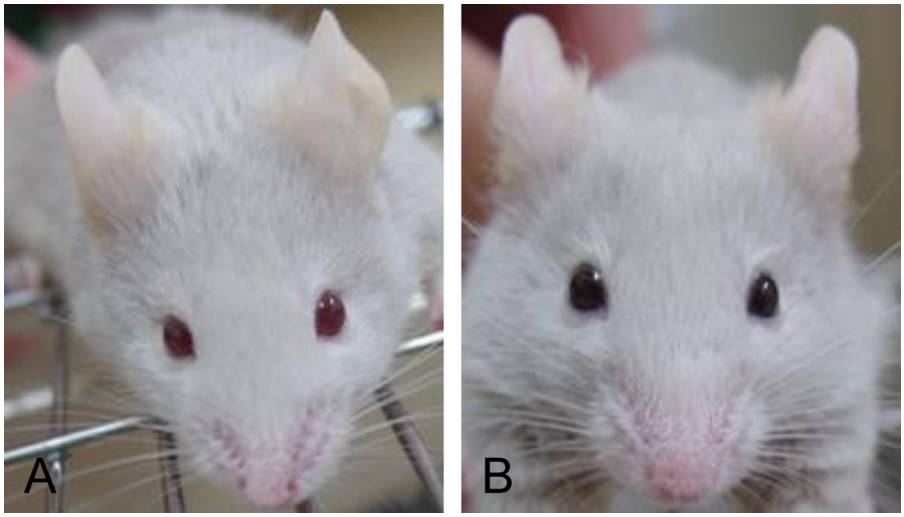

Fig. 1. Photographs of ordinary pink-eyed (A, B6;Cg-*Oca2*$^{p-cas}$/1Nga, 5-month-old) and novel black-eyed (B, B6;Cg-*Oca2*$^{p-cas}$/2Nga, 4-month-old) mutant (*Oca2*$^{p-cas}$/*Oca2*$^{p-cas}$) mice on the C57BL/6JJcl background. In the black-eyed mutant, the eyes gradually become black and the coat becomes darker. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)