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Histological Study of Oculocutaneous Albinism in the Korean Bitterling, *Acheilognathus signifer* (Osteichthyes; Cyprinidae)

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ABSTRACT The Korean bitterling, *Acheilognathus signifer* (Osteichthyes Cyprinidae), is an endemic and endangered species in Korea. During developmental stages, a small number of oculocutaneous albinism (with colorless body and eyeballs) were observed in the species. In order to investigate histological differences between normal and albinic bitterling, the dorsal skin and choroid-retina of the eyes were taken. The skin and eyes of normal and albino bitterling were similar in structure except for the presence or density of pigment cells. In normal bitterling, the epidermal melanocytes and dermal melanophores were abundantly developed in both the skin and epidermis of the eyes. But in the albino, the dorsal skin had few melanins, and the pigment cells over the choroid-retina pigment epithelium and iris of the eye were very small.

Key words : Korean bitterling, oculocutaneous albinism, melanocyte, melanophore, choroid-retina pigment epithelium

INTRODUCTION

The Korean bitterling, *Acheilognathus signifer* (Osteichthyes; Cyprinidae), is not only an endemic species distributed in the Han, Imjin, Daedong and Abrok River from Korea, but also an endangered species designated by the Ministry of Environment, Korea (Kim *et al*., 2005).

Recently, phenotypically conspicuous oculocutaneous albino-type bitterlings, about 300 individuals for 6 generations, have been made while carrying the artificial fertilization as way of protecting threatened freshwater fishes by National Fisheries Research & Development Institute (NFRDI), Korea. The albinism has been well known in human beings and other animals comprising the fishes (Vielkind *et al*., 1971; Koga and Hori, 1997; Fukamachi *et al*., 2001; Yoo *et al*., 2003; Lamoreux *et al*., 2005; Kang *et al*., 2007). This phenomenon may caused by negative-regulation of pigmentation of which the mechanism is not clearly characterized (Lin and Fisher, 2007).

Even though it has been known that the albinic individuals were phenotypically different from normal one in regard to less-developed coloration, there were not detailed histological reports about it. Therefore, we are histologically going to study overall morphology of skin and eyeball of *A. signifer*, especially regarding melanocyte and melanophore compared to normal bitterling.

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MATERIALS AND METHODS

Each 10 specimens in albino and normal bitterling, *A. signifer*, were used to carry out the histological study. The fragments of the dorsal skin and the eyes (choroid

Fig. 1. The Korean bitterling, *Acheilognathus signifer*. (A) normal bitterling; (B) albino bitterling. Bar indicates 2 cm.

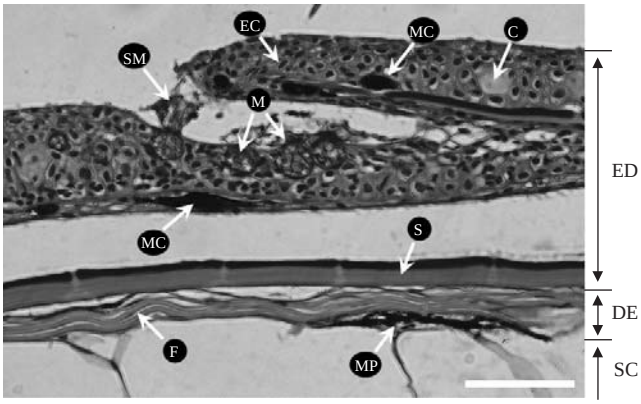

Fig. 2. The general morphology of the dorsal region in normal bitterling, *Acheilognathus signifer*. C, club cell; DE, dermis; EC, Epithelium cell; ED, epidermis; F, fibroblast; M, mucus cell; MC, melanocyte; MP, melanophore; S, scale; SC, subcutis; SM, secreted-mucous material. Bar indicates 50 μm. Hematoxylin-Eosin staining.

and iris) were dehydrated through as standard ethanol series to 100%, cleared in xylene and embedded in wax (Paraplast, Oxford). For histological observation 5 μm sections were cut using a microtome, deparaffinized and stained with hematoxylin-Eosin and Fontana-Masson staining which are specific for argentaffin melanin cells. The Axio imager. A1 (Carl Zeiss, Germany) and the Axio Vision (Ver. 4.5, Germany) were used for image capture and analysis.

Transmission electron microscopy (TEM) was also used. For this purpose, skin fragments were excised and prefixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2. Post-fixation was performed in 1% osmium tetroxide in the same buffer. After dehydration in a graded alcohol series, fragments were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed under EOL-1200EX transmission electron microscope.

RESULTS

1. External morphology

There were not any differences between normal and albinic bitterling in their overall shapes, but it showed clear differences in their colorations (Fig. 1). In the case of the normal bitterling, the skin showed most black, green and yellow color, whereas the albinic one looks like yellow or colorless white in appearance. Also the eyes of the normal bitterling has a conspicuous black pigments, but albinic one looks red.

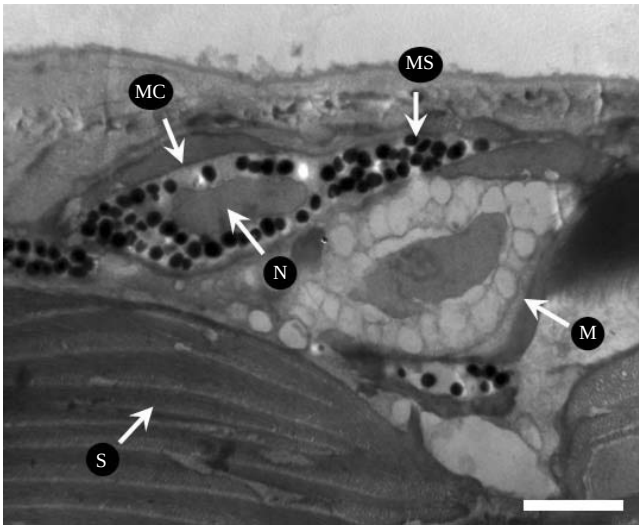

Fig. 3. TEM showing the epidermal melanocyte (MC) and enlarged mucus cell (M) of *Acheilognathus signifer*. Melanocyte contained nucleus (N) and numerous melanin granules (melanosomes; MS) within its cytoplasm, and transported out to epidermal matrix. Mucus cell located just above the scale (S) which it contained abundant glycoprotein granules within the cytoplasm and shown definitive nucleus and nucleolus. Bar indicates 2 μm.

2. Histological observation

1) Dorsal skin

Regardless of normal or albinic bitterling, the skin of *A. signifer* is composed distinctively of epidermis, dermis and subcutis layer (Fig. 2). The epidermis consisted of stratified squamous epithelium cells in about 6 rows and specialized two kinds of cells including small mucus cells and large club cells. In normal bitterling, pig-

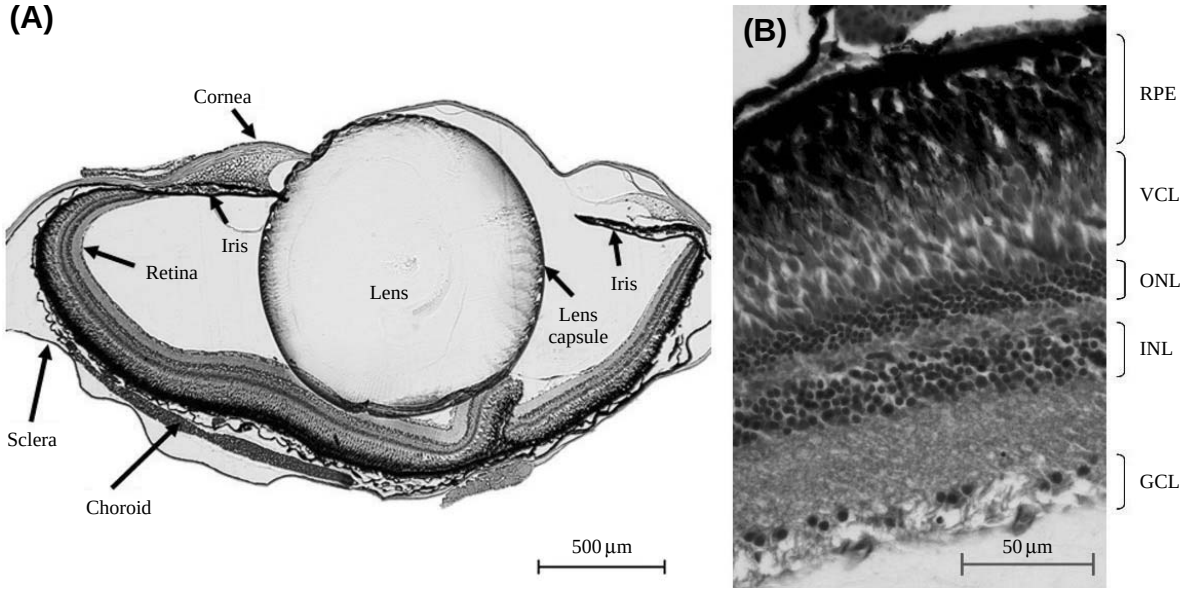

Fig. 4. The radial section of the eyeball (A) and magnification of retina (B) in normal bitterling, *Acheilognathus signifer*. RPE, retina pigment epithelium; VCL, visual cell layer; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Hematoxylin-Eosin staining.

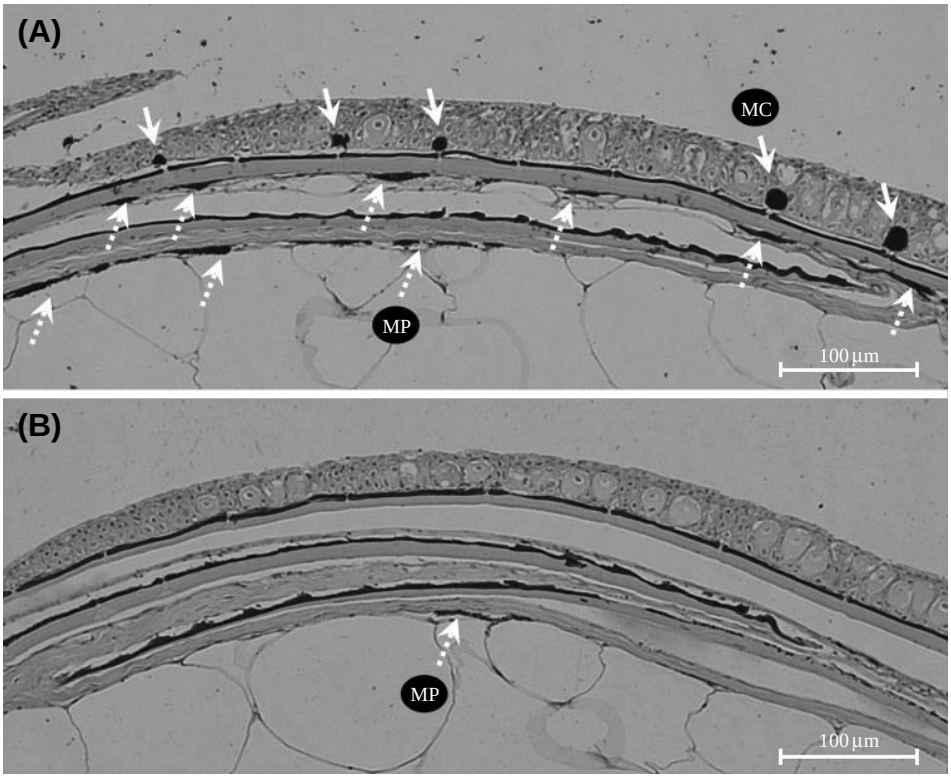

Fig. 5. Development of melanocytes and melanophores in the dorsal region of the normal (A) and albino (B) bitterling, *Acheilognathus signifer*. The pigment cells are stained with black as a result of accumulation of argent anion. Solid-arrows and dotted- arrows indicate melanocyte (MC) and melanophore (MP), respectively. Normal bitterling showed lots of melanin development, whereas albino showed melanin deficiency or poor development. Fontana-Masson staining.

ment cells (melanocytes) are situated just beneath or in the epidermis and they look like clusters consisted of large number of black or brown dots in light microscope. In TEM observation, the epidermal melanocyte contained melanin granules (melanosomes) within its cytoplasm and their dendritic processes extended through the epidermal layer (Fig. 3).

The dermis had scales and consisted mainly of dense connective tissue having the collagen fibers and collagen -generating fibroblast cells. In normal bitterling, many

sparsely distributed pigment cells (melanophores) were found below the basement membranes and also in the connective tissue pockets on the underside of the scales.

The subcutis was the innermost and thinnest layer of the skin and was situated in between the dermis and the muscle. A large number of nerves and blood vessels were found in this layer. In hematoxylin and eosin preparations, this layer invariably shows numerous empty spaces which are occupied by adipose cells.

2) Eyeball

In both normal and albinic bitterling, the eyeball was composed of lens, sclera, cornea, iris, choroid and retina (Fig. 4A). Inside of eyeball filled with sticky vitreous body. The retina can be divided into several layers; retina pigment epithelium, visual cell layer, outer nuclear layer, inner nuclear layer and ganglion cell layer (Fig. 4B).

3. Pigment cell between normal and albinic bitterling

1) Dorsal skin

The general morphology of the skin was the same in normal and albinic bitterling. In the normal bitterling, the pigment cells were abundant in the epidermis and dermis, and these pigment cells became more clear in Fontana-Masson staining (Fig. 5A). The number of melanocytes and melanophores in dorsal region was 9.3 ± 1.2 and 14.0 ± 5.3 per 1 mm length, respectively. On the other hand, the albino one had a few melanocytes and melanophores in the dorsal skin that it just showed 1 or 2 melanophores, but no melanocytes (Fig. 5B).

2) Choroid-retina pigment epithelium and iris

In the normal bitterling, the epidermis of retina and iris had far more pigment cell than those of normal skin in density of their pigment cells. A great number of pigment cells were distributed over choroid-retina pigment epithelium (Fig. 6A). In the albino bitterling, however, the pigmentation was not developed at all in the choroid -retina pigment epithelium (Fig. 6B) or very poor (Fig. 6C). In the iris epithelium of normal bitterling like choroid-retina pigment epithelium, the melanin pigment cells were predominantly developed in overall region (Fig. 7A), but vestigial in albino one which appeared as scattered or even clustered black blotches (Fig. 7B).

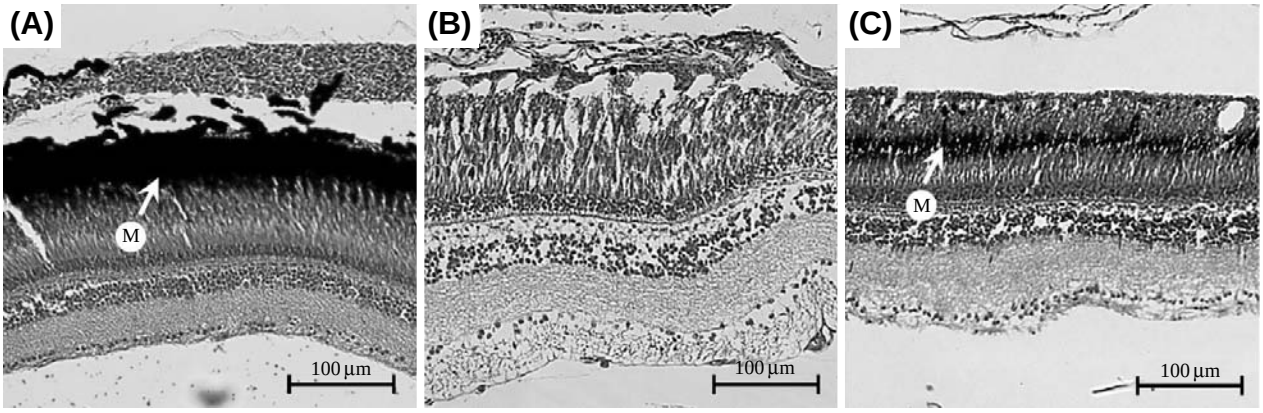

Fig. 6. Choroid-retina pigment epithelium of normal (A) and albino (B, C) of *Acheilognathus signifer*. Black regions are representative of melanin (M) as a result of accumulation of argent anion. Normal bitterling had lots of melanin pigment cells, whereas the albino one showed pigmentation deficiency or scarce presentation. Fontana-Masson staining.

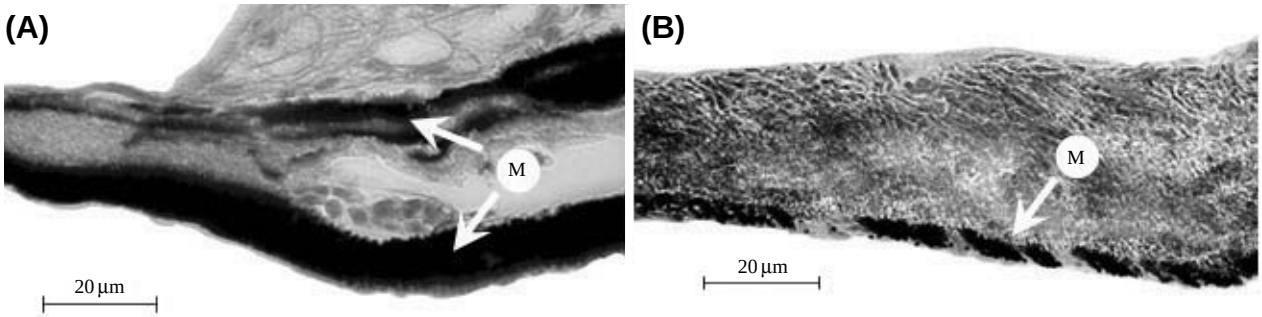

Fig. 7. Overview of iris structure from normal (A) and albino bitterling (B) of *Acheilognathus signifer*. Normal bitterling showed abundant melanin pigment cells (M) in its overall epithelium, but the pigmentation was so scarce in albino bitterling. Fontana-Masson staining.

DISCUSSION

Many literatures on oculocutaneous albinism have been tried to solve the mechanical reason concerning basic or clinical problems issued by the intrinsic manner of genetics and biochemistry (Koga and Hori, 1997; Fukamachi *et al*., 2001; Oetting *et al*., 2003; Lin and Fisher, 2007), furthermore focused on the accompanying commercial problems (Kang *et al*., 2007). The controlled factors like nutrition (especially DHA and Vitamin A whose defect can lead to a failure of normal pigmentation), lighting and substratum may play a significant role in abnormal pigmentation development of hatchery-reared flatfish (Bolker and Hill, 2000; Kang *et al*., 2007). However, there were no reports on histological approach.

Oculocutaneous albinism (OCA) is characterized by an inherited genetic disorder that has an inhibition in the pathway of melanin synthesis (Gronskov *et al*., 2007). The OCA have been well known from human beings and classified into 4 types as OCA1, OCA2, OCA3, OCA4 by the manner of proportional color representation (Okulicz *et al*., 2002; Gronskov *et al*., 2007). As *A. signifer* have red eyes and yellowish-white whole skin in coloration, it could be carefully classified *A. signifer* into criteria of "OCA1A", which the coding gene is *TYR* (Tyrosinase) seemed to be involved in melanocyte differentiation and melanosome formation (Lin and Fisher, 2007). In contrast to these categories, according to Koga and Hori (1997)'s classification, common defectiveness of the skin color and phenotypically distinctive red eyes in *A. signifer* would be categorized to i^1/i^1. But seeing the case of the choroid-pigment epithelium layer of retina region with a rare melanin pigment cells, it is more likely to be applied to i^4/i^4.

In this paper, we dealt with only dorsal skin and eyeball. But, in albinism of *A. signifer*, it is still questionable in regard that all skins covering the whole body and appendages like fins and barbels are having actually no pigment cells. Therefore, it will need to extend our research to the skin of appendages as well as other body regions in the future.

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묵납자루, *Acheilognathus signifer*의 Oculocutaneous Albinism에 대한 조직학적 연구

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요 약 : 한국고유종이자 멸종위기종인 묵납자루, *Acheilognathus signifer*의 종보존을 위한 인공발생 및 사육이 진행되는 동안 눈과 피부 전반에 걸쳐 색소의 발현이 결핍된 oculocutaneous albinism 개체가 출현하였다. 정상과 알비노간의 형태적 차이 여부를 알아보기 위해 등 피부와 안구의 맥락막-망막 조직을 적출하여 조직학적 분석을 실시하였다. 그 결과 일반적인 형태는 서로 차이가 없었으나 멜라닌색소의 발현에서 현저한 차이를 보였다. 정상 묵납자루는 등 피부의 표피와 진피, 맥락막-망막색소상피층 및 홍채에 많은 수의 멜라닌세포와 흑색소포가 존재한 반면, 알비노의 등 피부에서는 색소가 거의 존재하지 않았고 맥락막-망막색소상피층과 홍채에서는 매우 제한된 분포를 보였다.

찾아보기 낱말 : 묵납자루, 맥락막-망막색소상피층, 멜라닌세포, 흑색소포, oculocutaneous albinism