An increase in apoptosis and reduction in αB-crystallin expression levels in the lens underlie the cataractogenesis of Morioka cataract (MCT) mice

Abstract We examined the morphological changes in fibers, localization of apoptotic cells, and protein expression of αB-crystallin in the lens of Morioka cataract (MCT) mice, a novel cataract model. Using a scanning electron microscope, swollen lens fibers and enlarged spaces between lens fibers were observed in the lens of 3-week-old MCT mice. At 2 weeks of age (before cataract), the single-strand DNA (ssDNA)-positive (indicating apoptosis) cell ratio of the lens epithelium was significantly higher in MCT than in wild-type ddY mice. At 2 and 4 weeks of age, αB-crystallin protein expression of the lens in MCT mice was significantly lower than that in wild-type ddY mice. These findings suggest that increase in apoptosis and reduction in αB-crystallin level are involved in the cataractogenesis of MCT mice.

Key words Apoptosis · αB-Crystallin · Hereditary cataract mouse model · MCT mouse · Scanning electron microscopy

Introduction

Cataracts are the most common cause of blindness in the world.¹ Causes of cataracts include congenital defects, senility, metabolic disorders, and exposure to a variety of physical and chemical agents.² About 10–30% of childhood blindness cases are the result of congenital cataracts,³ and approximately one-quarter to one-third of congenital cataract cases are hereditary.⁴,⁵ Thus, the establishment of an animal model of cataracts will help elucidate the pathology underlying human cataractogenesis.⁷ Mice with cataracts were identified in a colony of ddY mice in our laboratory in 1997. The cataract mice were isolated from the colony, bred by random mating, and designated as Morioka cataract (MCT) mice. Cataracts in MCT mice are inherited in an autosomal recessive manner, and an opaque lens in these mice appears as a white pinpoint focus in the pink eyes of mice at 6 weeks of age.⁸

Ocular diseases, including cataracts, are influenced by metabolic disorders such as diabetic nephropathy.⁹ Therefore, in the present study, a blood analysis was performed to examine the relationship between the cataract in MCT mice and systemic disease. One of the most characteristic features of lens fiber cell differentiation is the loss of cell organelles,¹⁰ and apoptosis is observed in the developing lens fiber.¹¹ Moreover, crystallins are predominantly soluble proteins in the mammalian lens.¹² Up to 90% of the soluble protein in the postmitotic lens cells consists of proteins referred to as α-, β-, and γ-crystallin.¹³ α-Crystallins are molecular chaperones related to the small heat shock proteins¹⁴ and are considered to play an important role in maintaining the transparency of the lens.¹⁵ α-Crystallin consists of two subunits, αA and αB, existing in an approximately 3:1 ratio, and αB-crystallin increases resistance to several apoptotic inducers.¹⁶ Therefore, the present study designed to clarify the changes in morphology, apoptosis, and protein expression of αB-crystallin in the lens of MCT mice to elucidate the various cellular events underlying cataractogenesis.

Materials and methods

Animals and husbandry

MCT mice, a novel cataract strain derived from mice of the ddY strain, were used. Normal (wild-type) ddY mice were purchased from Japan SLC (Hamamatsu, Japan) and were
used as controls. All the animals were maintained under regulated room temperature (24°C ± 1°C), humidity (55% ± 5%) and lighting (from 0600 to 2000). Animals were given a commercial diet (CE-2; Clea, Osaka, Japan) and water ad libitum. Our studies were performed in accordance with the Guidelines for Animal Experimentation of Osaka Prefecture University, Japan.

Histological studies

For histological studies, MCT mice 3, 4, and 15 weeks old were used. Under isoflurane anesthesia, the mice were infused with heparin-saline, followed by 10% neutral buffered formalin. Eyes were then removed and immersed in the same fixative for 2 days. Eyes were dehydrated through a graded series of ethanol, soaked in butyl alcohol, and embedded in paraffin (Tissue Prep; Fisher Scientific, Fair Lawn, NJ, USA). Sections (4 μm) were cut in a plane perpendicular to the anteroposterior axis of the eye and stained with hematoxylin and eosin.

For scanning electron microscopic observations, 3-, 5-, and 15-week-old MCT mice were infused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The eyes were then removed and immersed in the same fixative for 24 h. The eyes were cut in a plane perpendicular to the anteroposterior axis of the eye. The lenses were removed, placed in the same fixative for 24 h, and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 24 h. The lenses were dehydrated in a graded series of ethanol, soaked in 3-methylbutyl acetate, and subjected to critical point drying. The lenses were coated with Pu-Pt ion coating and observed through the S-800 scanning electron microscope (Hitachi, Tokyo, Japan).

Immunohistochemical procedures

MCT mice 2 weeks old were used for immunostaining for single-strand DNA (ssDNA), and sections of eyes were prepared by the methods already described. After deparaffinization with xylene, the sections were transferred to distilled water through a graded series of ethanol and rinsed in phosphate-buffered saline. Immunostaining of ssDNA was accomplished by incubating the sections with rabbit anti-ssDNA antibody (Dako Cytomation, Glostrup, Denmark; 1:200, at 4°C, overnight). The sections were then incubated with biotinylated goat anti-rabbit IgG antibody (1:200) and an avidin-biotin-peroxidase complex (1:200) for 30 min, respectively. Finally, the sections were incubated with biotinylated goat anti-rabbit IgG antibody (1:200, at 4°C, overnight). The sections were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies diluted 1:1000 in blocking buffer. After washing in blocking buffer, blots were incubated for 30 min with horseradish peroxidase (HRP)-conjugated secondary antibodies diluted 1:1000 in blocking buffer. After washing in blocking buffer, the blot was developed using the ECL detection kit (Amersham Life Science, Buckinghamshire, UK). The integrated density of protein expression was determined using the NIH Image software. The relative protein expression levels of αB-crystallin were calculated as a percentage relative to the value of the expression of the α-tubulin protein.

Determination of ssDNA-positive cell ratio

Mitotically active cells are found just above the lens equator in the epithelial region (germinative region), and daughter cells from the germinative region move into the equatorial region, where they rapidly and greatly elongate and differentiate into secondary lens fibers. Thus, because dramatic events of lens differentiation are going on in the region just above the lens equator, the regions shown by the asterisks were used to determine the ssDNA-positive cell ratio lens epithelium (see Fig. 5a). The nuclei positive and negative for ssDNA were counted, and the ratio of positive nuclei to total nuclei was expressed as a percentage.

Blood chemical analysis

MCT mice 15 weeks old were used. Under isoflurane anesthesia, blood was collected after decapitation. Serum was analyzed on the day of blood draw for blood urea nitrogen (BUN), glucose, total cholesterol, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), calcium, albumin, and total bilirubin with an automatic chemistry analyzer system (Spotchem SP-4410; Kyoto Dai-ichi-Kagaku, Kyoto, Japan).

Statistics

The data were expressed as the mean ± SEM and analyzed with the Student’s t test. All P values less than 0.05 were identified as statistically significant.