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Regional Changes of AQP0-dependent Square Array Junction and Gap Junction Associated with Cortical Cataract Formation in the Emory Mutant Mouse

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Abstract

The Emory mutant mouse has been widely used as an animal model for human senile cataract since it develops late-onset hereditary cataract. Here, we focus on the regional changes of aquaporin-0 (AQP0) and connexins that are associated with the cortical cataract formation in the Emory mutant mice. Emory mutant and CFW wild-type mice at age 1 to 16 months were used in this study. By using an established photography system with dissecting microscopy, the opacities were first detected at the anterior or posterior lens center surface in Emory mice at age 7 months, and gradually extended toward the equator during the 16 months examined. Scanning EM verified that disorganized and fragmented fiber cells were associated with the areas of opacities within approximately 200 μ m from the lens surface, indicating that Emory mouse cataracts belong to the cortical cataracts. Freeze-fracture TEM further confirmed that cortical cataracts exhibited extensive wavy square array junctions, small gap junctions and globules. Immunofluorescence analysis showed that in contrast to the high labeling intensity of AQP0-loop antibody, the labeling of AQP0 C-terminus antibody was decreased considerably in superficial fibers in Emory cataracts. Similarly, a significant decrease in the labeling of the antibody against Cx50 C-terminus, but not Cx46 C-terminus, occurred in superficial and outer cortical fibers in Emory cataracts. Western blotting further revealed that the C-termini of both AQP0 and Cx50 in Emory cataracts were decreased to over 50% to that of the wild-type. Thus, this systematic study concludes that the Emory mouse cataract belongs to the cortical cataract which is due to regional breakdown of superficial fibers associated with formation of AQP0-dependent wavy square array junctions, small gap junctions and globules. The marked decreases of the C-termini of both AQP0 and Cx50 in the superficial fibers may disturb the needed interaction between these two proteins during fiber cell differentiation and thus play a role in the cortical cataract formation in Emory mutant mice.

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1. Introduction

The Emory mutant mouse strain was discovered and maintained by Kuck [Kuck et al., 1981] as an animal model for investigating human senile cataract. The Emory mutants develop late-onset light opacity around 7 months old and progressively turn into dense cataracts later at older age [Kuck et al., 1981; Kuck, 1990]. A number of biochemical and morphological studies have reported that many different changes are associated with the cataract formation [Kuck et al., 1981; Bhuyan et al., 1982; Swanson et al., 1985; Rathbun et al., 1986; Unakar et al., 1986; Lo and Kuck, 1987; DeNagel et al., 1988; Takemoto et al., 1988; Kuck and Kuck, 1989; Shi and Bekhor, 1992; Taylor et al., 1995; Reddy et al., 1999; Congdon et al., 2000; Sheets et al., 2002].

Despite these considerable studies, it is not yet certain whether the Emory mouse cataract belongs to the cortical or the nuclear cataract. The Emory mouse cataract was generally thought to be nuclear cataract because a hazy nucleus was detected when viewed from the anterior or posterior lens surface [Kuck et al., 1981; DeNagel et al., 1988; Kuck, 1990]. By using a slit-lamp microscope, a combination of the early cortical cataract and the late nuclear cataract has been reported [Takizawa and Sasaki, 1986]. However, by histopathological and electron microscopic examinations, Emory mouse cataract was found primarily associated with the cortical fibers and would be considered as the cortical cataract [Uga et al., 1988].

In this study, we have conducted a systematic examination of Emory mutant lenses to clarify the specific type of senile cataract in the Emory mutant mice. More importantly, during the course of this study, we have consistently observed unusual distributions of AQP0-dependent wavy square array junctions and small gap junctions in disorganized cortical fibers in Emory mice. We have thus focused on the specific regional changes of AQP0, Cx50 and Cx46 associated with the opacification in the Emory mouse cataract. Our results have clarified that the Emory cataracts belong to the senile cortical type based on the consistent presence of cellular breakdown only in the superficial and outer cortical regions in all aged lenses examined. In addition, the present study also reveals that the marked decreases of both C-termini of AQP0 and Cx50, but not Cx46, occur in the superficial and outer cortical fibers during formation of senile cortical cataract in the Emory mice.

2. Materials and methods

2.1. Animals and lens transparency photography

Emory mutant mouse breeders (strain name: Swiss Webster CFW-Em/J) were purchased from the Jackson Laboratory (Bar Harbor, Maine, U.S.A.). The wild-type mouse breeders (Swiss Webster CFW) were purchased from Charles River (Wilmington, MA, USA). Both breeders have been under a breeding program at the animal facility of Morehouse School of Medicine. A total of approximately 150 Emory mutant and wild-type mice from 1–16 months old were used for various experimental approaches in this study. Lenses were removed from freshly enucleated eyeballs of Emory mice and CFW controls of both genders at various age intervals, and collected in the PBS solution at RT. They were immediately determined and documented for their transparency under a dissecting microscopy system (Nikon SMZ800, Tokyo, Japan) equipped with a lens collecting glass (painted black) and a

digital camera (Nikon Coolpix5000, Tokyo, Japan) modified from the original design by Kuck [Kuck et al., 1981]. This modified microscopy system was enhanced with a high resolution objective lens and better light source (FS1000 Micro-Lite fluorescent electronic ring illuminator with new glare free "full spectrum" FS150 bulb, TEquipment.net, Long Branch, NJ) for capturing excellent contrasted images. Lenses were then fixed immediately in the fixative for the various experiments described below. The animals were treated in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research.

2.2. Scanning electron microscopy

Freshly isolated lenses of Emory mutant mice and controls at various ages were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3 at room temperature for 48–72 h. Each lens was properly orientated and fractured with a needle or sharp razor blade to expose the longitudinal configuration of fiber cells at the anterior, equatorial and posterior region of the lens (Fig. 1). Lens halves were then postfixed in 1% aqueous OsO₄ for 1–2 h at room temperature, dehydrated in graded ethanol and dried in a Samdri-795 critical point dryer (Tousimis Inc., Rockville, MD). Lens halves were oriented and mounted on specimen stubs and coated with gold/palladium in a Hummer VII sputter coater (Anatech Inc., Union City, CA). Micrographs were taken with a JEOL 820 scanning electron microscope at 10 kV (JEOL, Tokyo, Japan).

2.3. Histology microscopy

All freshly isolated lenses of Emory mutant mice and controls at various ages were fixed in an improved fixative containing 2.5% glutaraldehyde, 0.1 M cacodylate buffer (pH 7.3), 50 mM L-lysine and 1% tannic acid for 2 h at room temperature [Lo, 1988]. Each lens was then mounted on a specimen holder with superglue and cut into 200 µm slices with a Vibratome. Each lens was carefully oriented on the specimen holder such that either a cross- or longitudinal section of cortical fibers could be obtained initially with a Vibratome. Lens slices were then postfixed in 1% aqueous OsO₄ for 1 h at room temperature, rinsed in distilled water and stained en bloc with 0.5% uranyl acetate in 0.15 M NaCl overnight at 4°C. Tissue slices were dehydrated through graded ethanol and propylene oxide, and embedded in Polybed 812 resin (EMS, Hatfield, PA). Thick sections (1 µm) cut with a diamond knife were stained with 1% toluidine blue and examined with a Zeiss light microscope equipped with a digital camera.

2.4. Immunofluorescence labeling

Freshly isolated lenses of Emory mutant and wild-type mice were fixed in 4% paraformaldehyde-PBS (pH 7.3) for overnight at 4°C. They were washed for 2 × 30 min in PBS, infiltrated in 0.6 M sucrose-PBS for overnight at 4°C, and transferred to 1.15 M sucrose-PBS for second overnight at 4°C. Lenses were then embedded in OCT in plastic embedding molds for 2 h at RT and frozen in liquid nitrogen. Thick sections (20 µm thick) were cut with a cryostat at -20°C. Sections containing cortical and nuclear fibers were collected with ambient-temperature glass slides and stored in a -80°C freezer. The slides were later transferred to a -20°C freezer for overnight before use. For antibody labeling, sections were immersed in -20°C acetone for 2 min, washed in PBS at RT for 2 × 10 min,

and blocked in 2% BSA-PBS blocking solution for 1 h at RT. They were then incubated with (1) affinity purified rabbit AQP0 C-terminus polyclonal antibody (Alpha Diagnostic International, San Antonio, TX) at 1:200 dilution, (2) rabbit AQP0-loop polyclonal antibody [Johnson et al., 1991] (a gift from Ross Johnson of University of Minnesota, Minneapolis, MN; Purified rabbit Ab #7803 to peptide corresponding to positions 105–125 in bovine MIP26) at 1:200 dilution, (3) affinity purified rabbit Cx50 C-terminus polyclonal antibody at 1:500 dilution, and (4) affinity purified goat Cx46 C-terminus polyclonal antibody (M-19, SC-20861; Santa Cruz Biotechnology, Santa Cruz, CA) at 1:100 dilution with the blocking solution for 2 h at RT or overnight at 4°C. After washed in PBS for 2×10 min, sections were incubated with FITC-conjugated goat anti-rabbit IgG secondary antibody or with FITC-conjugated donkey anti-goat IgG secondary antibody at 1:200 dilutions (Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 h at RT. Some sections were also stained for fiber cell membranes with Rhodamine-conjugated Wheat Germ Agglutinin (WGA) (Vector Laboratories, Burlingame, CA) at 1:100 dilutions for 30 min at RT. All slides were rinsed in PBS, mounted with anti-photobleaching medium and examined with an Olympus or Zeiss LSM700 confocal microscope.

2.5. Freeze-fracture TEM and freeze-fracture replica immunogold labeling (FRIL)

Freshly isolated lenses of Emory mutant and wild-type mice were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.3) at RT for 2–4 hours. After washing in buffer, lenses were orientated to obtain sagittal (longitudinal) sections with a Vibratome, and slices were collected, marked serially from superficial to deep and kept separately. The slices were then cryoprotected with 25% glycerol in 0.1 M cacodylate buffer at RT for 1 hour and processed for freeze-fracture TEM according to our routine procedures [Biswas et al., 2009]. In brief, a single lens slice was mounted on a gold specimen carrier and frozen rapidly in liquefied Freon 22 and stored in liquid nitrogen. Cryofractures of frozen slices were made in a modified Balzers 400T freeze-fracture unit, at a stage temperature of -135°C in a vacuum of approximately 2×10^{-7} Torr. The lens tissue was fractured by scraping a steel knife across its frozen surface to expose fiber cell membranes. The fractured surface was then immediately replicated with platinum (~2 nm thick) followed by carbon film (~25 nm thick). The replicas, obtained by unidirectional shadowing, were cleaned with household bleach and examined with a JEOL 1200EX TEM.

For freeze-fracture replica immunogold labeling (FRIL), we followed our routine procedures [Biswas et al., 2009]. In brief, freshly isolated lenses of Emory mutant and wild-type mice were lightly fixed in 0.75% paraformaldehyde in PBS for 30–45 min at RT, and then cut into 300 μm slices with a Vibratome to make freeze-fracture replicas. One drop of 0.5% parlodion in amyl acetate was used to secure the integrity of the whole piece of a large replica during cleaning and immunogold labeling procedures. The replica was digested with 2.5% sodium dodecyl sulfate, 10 mM Tris-HCl, 30mM sucrose, pH 8.3 (SDS buffer) at 50°C until all visible attached tissue debris was removed from the replica. The replica was then rinsed with PBS, blocked with 4% BSA-0.5% teleostean gelatin in PBS for 30 min and incubated with (1) rabbit AQP0-loop polyclonal antibody (a gift from Ross Johnson of University of Minnesota, Minneapolis, MN; Purified rabbit Ab #7803 to peptide corresponding to positions 105–125 in bovine MIP26), (2) rabbit anti-AQP0 C-terminus

polyclonal antibody (Alpha Diagnostic International, San Antonio, TX), and (3) affinity purified rabbit Cx50 C-terminus polyclonal antibody, all at 1:10 dilution for 1 h at RT. The replica was washed with PBS and incubated with 10 nm Protein A gold (EY Laboratories, San Mateo, CA) at 1:50 dilution for 1 h at RT. After rinsing, the replica was fixed in 0.5% glutaraldehyde in PBS for 10 min, rinsed in water, collected on a 200-mesh Gilder finder grid, rinsed with 100% amyl acetate for 30 s to remove paraloidion and viewed with a JEOL 1200EX TEM.

Quantitative analysis was carried out to determine the changes in the binding of Cx50 C-terminus antibody on gap junction plaques in superficial fibers in Emory mutant and wild-type lenses. The micrographs at 30,000 $\times$ were taken randomly from flat cell membranes from three replicas prepared from three different animals. Each membrane area (μm^2) was measured with the Zeiss AxioVision LE 4.4 on PC, and the number of gold particles per unit membrane was counted manually with a cell counter. The number of particles per μm^2 membrane area was calculated. Statistical comparisons of the mean percentage of gold particles from Emory mutant and wild-type lenses were made by T-test using the software SPSS 14.0 (SPSS Inc.). A $P < 0.05$ was considered significant.

2.6. Western blot analysis

Lens membrane protein preparation was performed as previously described [Jiang and Goodenough, 1996]. Briefly, tissue lysates were prepared by homogenizing adult lenses of wild-type and Emory mice in lysis buffer (5mM Tris, 5mM EDTA, 5 mM EGTA, 250 mM sucrose and proteases inhibitors). The lens lysates were centrifuged at 100,000 $\times$ g at 4°C for 30 min. The pellets were resuspended in 20 mM NaOH and centrifuged again at 100,000 $\times$ g at 4°C for 30 min. The pellets were resuspended and protein concentrations were determined using the Micro BCA Protein Reagent assay kit (Pierce, Rockford, IL). Thirty micrograms of protein were loaded, separated on a 10% SDS-PAGE, and transferred to a nitrocellulose membrane. The membranes were blotted with affinity-purified antibodies for mouse Cx50 (1:1000 dilutions), mouse MIP (1:300 dilutions) or β -actin (1:5000 dilutions). Secondary antibodies conjugated with horseradish peroxidase, anti-rabbit antibody (1:5000 dilutions) for mouse Cx50 and AQP0, and anti-mouse antibody (1:5000 dilutions) for β -actin, were used to detect primary antibodies. The signals were detected using a chemiluminescence reagent kit (ECL) and X-OMAT AR films.

3. Results

3.1. Development of lens opacities and structural changes in different superficial cortical regions of Emory mutant mice

By using the dissecting microscopy system (see Methods) modified from Kuck [Kuck et al., 1981], we first detected the cortical cataracts at age late 7 months in all Emory mouse lenses examined (Fig. 1). The opacities were initiated from the central region of anterior or posterior superficial cortex and gradually extended to the equatorial regions at the advanced ages (Fig. 1B and C). Before the opacities extended to the equator, the distinct small transparent zone was clearly seen enclosing the entire lens equator (Fig. 1B and C). This limited (restricted) small transparent zone was due to the presence of normal superficial

fibers seen at the equatorial regions while other cortical fibers were already damaged at the anterior or posterior cortex in some Emory cataracts (Fig. 1E–G). At the advanced age, the severe damaged fibers were seen in the equatorial superficial cortex as viewed with SEM (Fig. 1H and I) and light microscopy (Fig. 1J). In the end, the cortical cataract appeared as a shell (zone) of opacity in the superficial and outer cortices within approximately 200 μm deep from the lens surface.

3.2. Surface structural changes of cortical fibers in Emory mouse cataract and wild-type lens

For consistent comparisons between Emory mutant and wild-type lenses, all SEM micrographs were systematically taken from the equatorial superficial cortex toward deep cortex of the lenses (Fig. 2A–H). The systematic SEM examination showed the distinct zone of wavy ridge-and-valleys only in deep cortex in the wild-type lens (Fig. 2A–D). With increasing ages after 7 months, superficial cortical fibers near the equator became disorganized, swollen and fragmented within approximately 200 μm deep from the lens surface in the Emory cataracts (Fig. 2E). These cells were covered with extensive wavy ridge-and-valley membrane surfaces in the damaged superficial regions (Fig. 2E). In addition, similar ridge-and-valley surfaces were also distributed in a narrowed distinct zone of intact fibers (approximately 250 μm deep) immediately below the damaged superficial fibers in all Emory cataracts examined (Fig. 2F). It was noted that this narrow zone of intact cortical fibers with distinct wavy ridge-and-valley surfaces actually appeared earlier (i.e., at age 6 months) than those shown in the damaged superficial fibers (Fig. 2E). Beyond this region, all underlying deeper cortical fibers in the Emory cataracts (Fig. 2G and H) were morphologically similar to those in the wild-type (Fig. 2C and D).

A representative profile in the opacity regions in equatorial cortex showed that in addition to the formation of wavy ridge-and-valley membrane surfaces (Fig. 3A–C), there were many globules of various sizes (Fig. 3B–D) in disorganized and fragmented fibers of the Emory cataracts.

3.3. Changes on wavy square array junctions in Emory mouse cataracts

The wavy square array junctions of the wild-type controls exhibited the orthogonal arrangements of intramembrane particles (6.6 nm) or pits along the valleys or sides of the junctions in the deep cortical fibers (Fig. 4A and B). Freeze-fracture immunogold labeling showed that the 10-nm gold particles for AQP0 C-terminus antibody were sparsely distributed along the valleys or sides of wavy square array junctions in the deep cortical fibers (Fig. 4C). In the Emory cataracts, freeze-fracture TEM also showed the typical orthogonal particles (6.6 nm) or pits associated with the valleys or sides of the wavy square array junctions in disorganized or damaged superficial fibers (Fig. 5A and B). In addition, many globule structures with either the smooth surface or intramembrane particles were also regularly distributed in the damaged superficial fibers in Emory cataracts (Fig. 5B and C). We conducted immunogold labeling of both AQP0-loop and AQP0 C-terminus antibodies in Emory cataracts to reveal the possible difference in disorganized or damaged superficial fibers. Figure 5D shows that the 10-nm gold particles for the AQP0-loop antibody were regularly distributed along the wavy square array junctions in superficial fibers of Emory

cataracts. In contrast, the gold particles for the AQP0 C-terminus antibody were sparsely distributed along the valley or sides of wavy square array junctions in the superficial fibers (Fig. 5E). The quantitative comparison between these two antibodies for their different labeling densities was not conducted in this study due to the difficulty in obtaining an accurate measurement for the highly undulating nature of the junction membrane surfaces.

Figure 6 shows immunofluorescence labeling of AQP0 antibodies in wild-type and Emory mouse lenses for the low magnification comparison. While the AQP0 C-terminus antibody was labeled strongly in the superficial fibers in the wild-type (Fig. 6A and B), the labeling of AQP0 C-terminus antibody was specifically decreased in the superficial fibers in the Emory mouse cataracts (Fig. 6C and D). In contrast, the AQP0-loop antibody (Fig. 6E and F) was labeled in a high intensity in the superficial fibers, indicating that AQP0 C-termini indeed underwent premature truncation in the superficial fibers in Emory cortical cataracts.

3.4. Differential changes on gap junction proteins in Emory mouse cataracts

Figure 7 shows that immunofluorescence labeling of antibodies against Cx50 C-terminus and Cx46 C-terminus differ significantly in cortical fibers in Emory mouse cataracts. The signal for Cx50 C-terminus antibody showed a dramatic decrease from superficial to outer cortical fibers in Emory cataract (Fig. 7C and D) as compared with the wild-type (Fig. 7A and B). In contrast, the Cx46 C-terminus antibody was labeled with the same intensity in superficial and outer cortical fibers in Emory mouse cataract (Fig. 7H–J). Similarly, the labeling intensity for Cx46 C-terminus antibody was also same in superficial and outer cortical fibers in the wild-type lens (Fig. 7E–G). However, it is not known whether the decrease in the Cx50 C-terminus labeling was due to the premature cleavage or the decrease in the total Cx50 protein. This issue is not determined because the Cx50-loop antibody is currently not available for this study.

Despite a significant decrease in Cx50 C-terminus labeling in superficial and outer cortical fibers, freeze-fracture TEM showed that gap junction plaques were regularly distributed in both superficial and deeper cortical fibers, and were generally small in size in Emory mouse cataracts studied (Fig. 8A and B). Importantly, freeze-fracture immunogold labeling showed that the gold particles for Cx50 C-terminus were greatly decreased on gap junctions of flat cell membranes or globular membranes in cortical fibers in the Emory cataract (Fig. 8C–E) as compared with the control (Fig. 8F). Quantitative analysis on electron micrographs showed that gold particles representing the Cx50 C-termini of fiber gap junctions in Emory mouse cataracts were indeed significantly less than those of the wild-type (Fig. 8G) (***, $P < 0.001$). The analysis included a total of 2415 gold particles that were counted from micrographs taken randomly from three large replicas of wild-type and of Emory mutant lenses.

3.5. Western blot analysis of AQP0 C-terminus and Cx50 C-terminus

To validate the immunolabeling data presented above, Western blotting analysis using corresponding anti-C-terminus antibodies indeed confirmed that signals for both AQP0 and Cx50 in Emory cataracts were decreased to over 50% to that of the wild-type (Fig. 9).

4. Discussion

This study demonstrates that Emory mutant cataracts belong to senile cortical cataracts rather than the nuclear cataracts described in the early reports [Kuck et al., 1981; Kuck and Kuck, 1983; Takizawa and Sasaki, 1986; Kuck, 1990]. The identification of cortical cataract in the Emory mutant mice is achieved by the systematic, correlative examinations with the opacity photographs, histology, scanning and freeze-fracture electron micrographs. The Emory cataracts show significant disorganization, swelling and breakdown of fiber cells in superficial and outer cortical regions up to approximately 200 μm from the equatorial surface from all lenses at ages 7 to 16 months examined (Fig. 1). This finding supports the histopathological observations by Uga [Uga et al., 1988].

One of the unique changes in the Emory cortical cataract is the formation of wavy square array junctions (ridge-and-valleys) in the disorganized and fragmented superficial cortical fibers (Figs. 2, 3 and 5). The major protein of square array junctions is a truncated water channel protein, aquaporin-0 (AQP0) [Simon et al., 1982; Zampighi et al., 1982; Costello et al., 1989; Zampighi et al., 1989]. Typically, the AQP0 C-terminus undergoes proteolytic cleavages during maturation and aging of fiber cells that result in the formation of a truncated new 22–24 kD molecular weight membrane protein in the normal lens [Horwitz et al., 1979; Roy et al., 1979; Alcala et al., 1980; Takemoto et al., 1986]. This age-related cleavage of AQP0 C-terminus is directly associated with the formation of AQP0-dependent wavy square array junctions in the deep cortex and nucleus of the lenses in various species examined [Zampighi et al., 1982; Lo and Harding, 1984; Costello et al., 1985; Lo and Kuck, 1987; Costello et al., 1989; Zampighi et al., 1989]. In the present study, the wavy square array junctions are uniquely observed in the superficial cortical fibers of Emory cataracts, which exhibit the same structural characteristics as those seen in the deep cortical fibers of transparent lenses examined with SEM, freeze-fracture TEM and immunogold labeling (Figs. 2, 4 and 5). These results suggest that the wavy square array junctions (approximately 150 nm in each junctional unit) per se do not cause lens opacity in Emory mouse cataracts. Rather, the breakdown of superficial fibers and formation of numerous large globules are responsible for the opacification (Figs. 1, 3 and 5). The cause of fiber cell breakdown in superficial cortex is not known.

Although the cleavage of AQP0 C-terminus in lens fibers has been previously reported in Emory cataracts [Takemoto et al., 1988; Lo and Kuck, 1990], this study specifies that the premature cleavage of AQP0 C-termini occurs in the superficial cortical fibers as demonstrated with the positive immunolabeling of AQP0-loop antibody in the same region (Figs. 5 and 6). This specific regional change would be more directly associated with the formation of cortical cataracts in Emory mice (Figs. 1 and 2). The cleavage of AQP0 C-terminus can be accelerated by the increase of calcium in the fiber cells [Alcala et al., 1987; Peracchia and Girsch, 1989; Ma et al., 2005; Shih et al., 2006]. An increased accumulation of calcium during cataract formation in Emory cataract has been reported [Kuck and Kuck, 1989]. In addition, recent in vitro studies suggest that aquaporin-0 (square array) junctions are formed with closed water channels after cleavage of AQP0 C-termini [Gonen et al., 2004a; Gonen et al., 2004b; Gonen and Walz, 2006; Engel et al., 2008]. It is speculated in

this study that premature cleavage of AQP0 C-terminus may cause closure of water channels in superficial fibers which may cause subsequent structural changes and opacification.

It is interesting that only Cx50 C-terminus, but not Cx46 C-terminus, is markedly decreased in superficial and outer cortical fibers in Emory cataracts (Figs. 7, 8 and 9). The significant decrease of Cx50 C-terminus can be a result of either dramatic premature cleavage of its C-terminus or massive deduction of total Cx50 protein or both in superficial and outer cortical fibers. Since the Cx50-loop antibody is not available for this study, we have not been able to validate this question. The co-localization of AQP0 and Cx50 in several specialized lens fiber membrane domains such as gap junctions and interlocking ball-and-socket domains has been reported [Gruijters, 1989; Dunia et al., 1998; Zampighi et al., 2002]. Recent studies have further demonstrated that AQP0 enhances gap junction coupling via its cell adhesion function and interaction with Cx50 [Liu et al., 2011; Kumari et al., 2013]. More importantly, the direct interaction between the C-terminus of AQP0 and the intracellular loop of Cx50 during fiber cell differentiation has been demonstrated [Yu and Jiang, 2004; Yu et al., 2005]. Taken together, the results of this study suggest that the significant decreases of the C-termini of AQP0 and Cx50 in superficial and outer cortical fibers may impair the needed interactions between these two proteins during fiber cell differentiation and thus play a role in the formation of cortical cataract in Emory mice.

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Highlights

- Emory mouse cataract belongs to the senile cortical type.
- Damaged fibers form wavy square array junctions, small gap junctions and globules.
- The C-termini of AQP0 and Cx50 are decreased markedly in damaged cortical fibers.
- These changes may disrupt the needed interaction of AQP0 and Cx50 for transparency.

Figure 1.

Transparency photographs show transparent lenses at age 6 months (A), cortical cataracts at 9 months (B) and 12 months old (C) in Emory mutant mice. The dense cortical opacity is often observed at the anterior or posterior center surface without reaching the equator, as marked by two opposite single arrows (B, C). The double arrows indicate the reaching of lens opacity to the equatorial surface in an Emory cataractous lens at age 12 months (C). SEM reveals the intact cortical fibers (arrow) at the equatorial region in Emory mouse lenses at ages 6, 7, 9 and 12 months, and the damaged fibers (asterisk) near the anterior or posterior

sutures (E–G), and the equator (F–G) at ages 7, 9 and 12 months. Damaged fibers (asterisk) at the lens equator (box) at ages 9 and 12 months in F and G were shown at higher magnifications in H and I, respectively. Damaged fibers were also clearly seen at the anterior superficial cortex of a 16-month-old Emory cataract with thick-section light microscopy (J). e, lens epithelium. Scale bars: D–G = 200 μm ; H = 20 μm ; I = 50 μm ; J = 20 μm .

Figure 2.

SEM shows regional differences between transparent wild-type lens (A–D) and Emory mouse cataract (E–H) at 9 months of age. Micrographs shown were taken from approximately 100 μm to 600 μm deep from the lens equatorial surface. Disorganized or damaged fiber cells are seen primarily in superficial cortical regions in Emory cataracts (E). Noticeably, the distinct wavy ridge-and-valley surfaces are regularly formed in the intact outer cortical fibers only in Emory cataracts (F). The similar undulating ridge-and-valley

surfaces are normally distributed in deep cortical fibers in both wild-type and Emory cataractous lenses (D, H). Scale bars: A–H = 1 μm .

Figure 3. SEM displays a representative profile of damaged superficial fiber cells with different surface patterns including wavy ridge-and-valleys and globules of various sizes in Emory mouse cataracts at various ages. Scale bars: A–D = 1 μm.

Figure 4.

Freeze-fracture and immunogold labeling analyses of wavy square array junctions in deep cortex of wild-type lenses. Wavy square array junctions show the well-registered 6.6-nm square array particles (arrows) along the sides or valleys of the junctions (A, B). Freeze-fracture immunogold labeling shows that 10-nm gold particles representing the AQP0 C-terminus antibody (C) are localized along the valley or sides of wavy square array junctions. pf, protoplasmic face; ef, exoplasmic face. Scale bars: 100 nm.

Figure 5.

Freeze-fracture TEM and immunogold labeling analyses of wavy square array junctions and globules in superficial cortex in Emory mouse cataracts. The well-ordered 6.6-nm square array particles (arrows) are distributed along the sides or valleys of wavy square array junctions (A). They exhibit the same structural features as those in the deep cortex of the wild-type lenses (see Fig. 4). In addition, the wavy square array junctions often co-exist with clusters of globules (open arrows) which display smooth surface, intramembrane particles, or small gap junction plaque (arrow) (B, C). Freeze-fracture immunogold labeling shows

that 10-nm gold particles for the AQP0-loop antibody are regularly distributed along the valleys or sides of wavy square array junctions (D). In contrast, the 10-nm gold particles for the AQP0-C terminus antibody are sparsely distributed along the valleys or sides of wavy square arrays (E). pf, protoplasmic face; ef, exoplasmic face. Scale bars: 100 nm.

Figure 6.

A comparative immunofluorescence labeling for the AQP0 C-terminus and the loop antibodies in wild-type and Emory cataractous lenses at age 9 months. In contrast to the full labeling in the wild-type (A, B), the labeling for AQP0 C-terminus is specifically reduced or absent in the superficial cortical regions in the Emory mouse cataract (C, D). However, the AQP0-loop antibody shows the full labeling in the Emory mouse cataract (E, F), suggesting that C-terminus of AQP0 is prematurely cleaved in the superficial cortex. The DIC images (B, D, F) were used here for the fiber-cell region identification. C, lens capsule. Scale bars: A–D = 50 μ m.

Figure 7.

Immunofluorescence labeling for the C-terminus antibodies of both Cx50 and Cx46 in wild-type and Emory mouse cataractous lenses at age 9 months. While the labeling for the Cx50 C-terminus antibody is regularly distributed in both superficial and outer cortical regions in wild-type lens (A, B), it is markedly limited in the minor superficial cortical region in the Emory mouse cataract (C, D). In contrast, the labeling for Cx46 C-terminus antibody is regularly distributed in both superficial and outer cortical regions in both wild-type (E–G) and Emory mouse cataract (H–J). The DIC images (B, D) and Rhodamine-WGA (F, I) are used here for fiber-cell region identification. Scale bars: A–D = 50 μm . c, lens capsule; scf, superficial cortical fibers; ocf, outer cortical fibers. Scale bars: E–J = 20 μm .

Figure 8.

Freeze-fracture TEM reveals the presence of small gap junctions in superficial (A) and deeper cortical fiber cells (B). Immunogold labeling shows the sparse distribution of 10-nm gold particles for the Cx50 C-terminus antibody in gap junction plaques on flat membranes (C, D) and an isolated globule (E). In contrast, a large number of gold particles are distributed on a gap junction plaque in wild-type cortical fiber cells (F). Quantitative analysis shows that the distribution of Cx50 C-terminus labeling in gap junction plaques in the Emory cataract is significantly less than that of the wild type (G). A total of 2415 gold

particles were counted from micrographs taken randomly from three large replicas of wild-type and Emory mutant lenses. Scale bars: A–B = 200; C–F = 100 nm.

Figure 9.

Western blotting shows that Cx50-C terminus and AQP0-C terminus are decreased in Emory mouse cataracts (E) to over 50% to that of the wild-type (W). Three groups of membrane preparations from adult lenses of wild type (W) and Emory (E) mice were analyzed by western blotting using antibodies specific for Cx50 C-terminus and AQP0 C-terminus. β -actin was used as a protein loading control.