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Autophagy and UPR in alpha-crystallin mutant knock-in mouse models of hereditary cataracts☆

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ABSTRACT

Background: Knock-in mice provide useful models of congenital and age-related cataracts caused by α-crystallin mutations. R49C αA-crystallin and R120G αB-crystallin mutations are linked with hereditary cataracts. Knock-in αA-R49C +/− heterozygotes develop cataracts by 1–2 months, whereas homozygote mice have cataracts at birth. The R49C mutation drastically reduces lens protein water solubility and causes cell death in knock-in mouse lenses. Mutant crystallin cannot function as a chaperone, which leads to protein aggregation and lens opacity. Protein aggregation disrupts the lens fiber cell structure and normal development and causes cell death in epithelial and fiber cells. We determined what aspects of the wild-type phenotype are age-dependently altered in the mutant lens.

Methods: Wild-type, heterozygote (αA-R49C +/−), and homozygote (αA-R49C +/+) mouse lenses were assessed pre- and postnatally for lens morphology (electron microscopy, immunohistochemistry), and autophagy or unfolded protein response markers (immunoblotting).

Results: Morphology was altered by embryonic day 17 in R49C +/+ lenses; R49C +/− lens morphology was unaffected at this stage. Active autophagy in the lens epithelium of mutant lenses was indicated by the presence of autophagosomes using electron microscopy. Protein p62 levels, which are degraded specifically by autophagy, increased in αA-R49C mutant versus wild-type lenses, suggesting autophagy inhibition in the mutant lenses. The unfolded protein response marker XBP-1 was upregulated in adult lenses of αB-R120G +/+ mice, suggesting its role in lens opacification.

Conclusions: Mutated crystallins alter lens morphology, autophagy, and stress responses.

General significance: Therapeutic modulation of autophagic pathways may improve protein degradation in cataractous lenses and reduce lens opacity. This article is part of a Special Issue entitled Crystallin Biochemistry in Health and Disease.

1. Introduction

The crystallin protein family accounts for 90% of lens proteins and plays a key role in lens transparency [1,2]. Point mutations in the genes encoding α-, β-, and γ-crystallins cause hereditary human cataract formation at birth or at an early age [3–5]. Alpha-crystallin is an oligomer of two polypeptides, αA- and αB-crystallin, that are expressed in lens epithelial and fiber cells. Human patients harboring single point mutations in αA- and αB-crystallin genes develop hereditary cataracts [6–8]. The distribution of α-crystallin in human lenses changes with aging [9,10]. There is strong correlation between loss of α-crystallin from the lens soluble protein fraction and increased light scattering and lens opacification in human cataracts [9]. Genetically defined cataracts are attractive model systems for studying the mechanisms of cataract formation. Age-related cataracts are very heterogeneous and multifactorial in origin. Cataracts caused by specific mutations in crystallin genes occur earlier in life and can be studied in animal models. Cataract-causing mutations identified in human αA-crystallin include the R49C [11], R116C [8], R116H [12], G98R [13], R54L [14], R21Q [15], and W9X mutations [16]. The αA-R49C mutation causes protein aggregation, cell death, and mislocalization of the mutant protein to the cellular nucleus. The mutant protein also forms crosslinks with other crystallins in vitro. Mutations in the αB-crystallin gene that cause human cataracts include the R120G, R11H, and P20S mutations [7,17,18]. The αB-R120G mutant protein also forms high-molecular weight aggregates, and knock-in mice have been generated to understand the mechanism of cataract formation by this mutant [19].

Studies in the past two decades have demonstrated the importance of genetic factors in the etiology of age-related cataract [20,21]. Functional studies of hereditary cataract formation in animal models can improve our understanding of the etiology of age-related cataracts [22,23]. To understand disease etiology in hereditary cataracts, we have used embryonic stem cell-based technologies to generate knock-in mice

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expressing α-crystallin proteins containing either the αA-R49C or αB-R120G mutation. These two mutations are associated with human autosomal dominant hereditary cataracts. Point mutation gene knock-in mice have a single-nucleotide mutation in a gene that does not ablate the gene but merely changes its function. Knock-in mice offer the advantage of comparing wild type, heterozygous, and homozygous mice, and permit studying the effects of gene dosing in vivo. Knock-in mice have been used to study the role of specific mutations in connexin and αA- and αB-crystallin genes that cause cataract pathology [19,24,25].

2. Materials and methods

Experiments using mice were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. All animal procedures were performed by the Washington University Mouse Genetics Core, following protocols approved by the Washington University Animals Studies Committee. Mice were euthanized via CO_2 asphyxiation followed by cervical dislocation. For neonatal mice, decapitation was used. Eyes were enucleated and lenses were extracted on ice in Dulbecco's phosphate-buffered saline (PBS) containing protease inhibitors (1:1000 vol/vol of protease inhibitor cocktail; Sigma-Aldrich, St. Louis, MO). Lenses were manually homogenized to extract water-soluble proteins, and homogenates were centrifuged at 20,800 × *g* for 30 min to separate soluble and insoluble fractions. After withdrawing the soluble supernatant fraction, the remaining protein pellet was dissolved in 1 × PBS containing 8 M urea. Bradford assays provided sample protein concentrations for uniform gel loading. Western blotting used primary antibodies against β-actin (Sigma A1978), p62 (Sigma P0067), and XBP-1 (Santa Cruz Biotech), at dilutions of 1:500–1:1000 in either Odyssey Blocking Buffer (LI-COR, Inc., Lincoln, NE) or 5% non-fat dried milk in Tris-buffered saline. After washing, IRDye secondary antibodies (LI-COR) were used to image the blots on an Odyssey SA Infrared Imaging System (LI-COR); band intensities were quantified using Odyssey 3.0 software. Histological and immunocytochemical analysis was performed on lens sections according to the methods described previously [26,27]. Transmission electron microscopy was performed by our Molecular Microbiology Imaging Core facility [27].

3. Results and discussion

3.1. αA-crystallin mutation affects embryonic histology at embryonic day 17

Human patients heterozygous for the αA-R49C mutation in αA-crystallin develop cataracts at birth or during infancy [11]. The αA-R49C heterozygous mice that mimic the heterozygosity of human patients have one mutant allele and one wild-type allele. Knock-in heterozygote mice exhibit minor lens defects by 2 months of age, including reduced protein solubility and increased lens opacification. Homozygous αA-R49C knock-in mice that express only mutant αA-crystallin have more

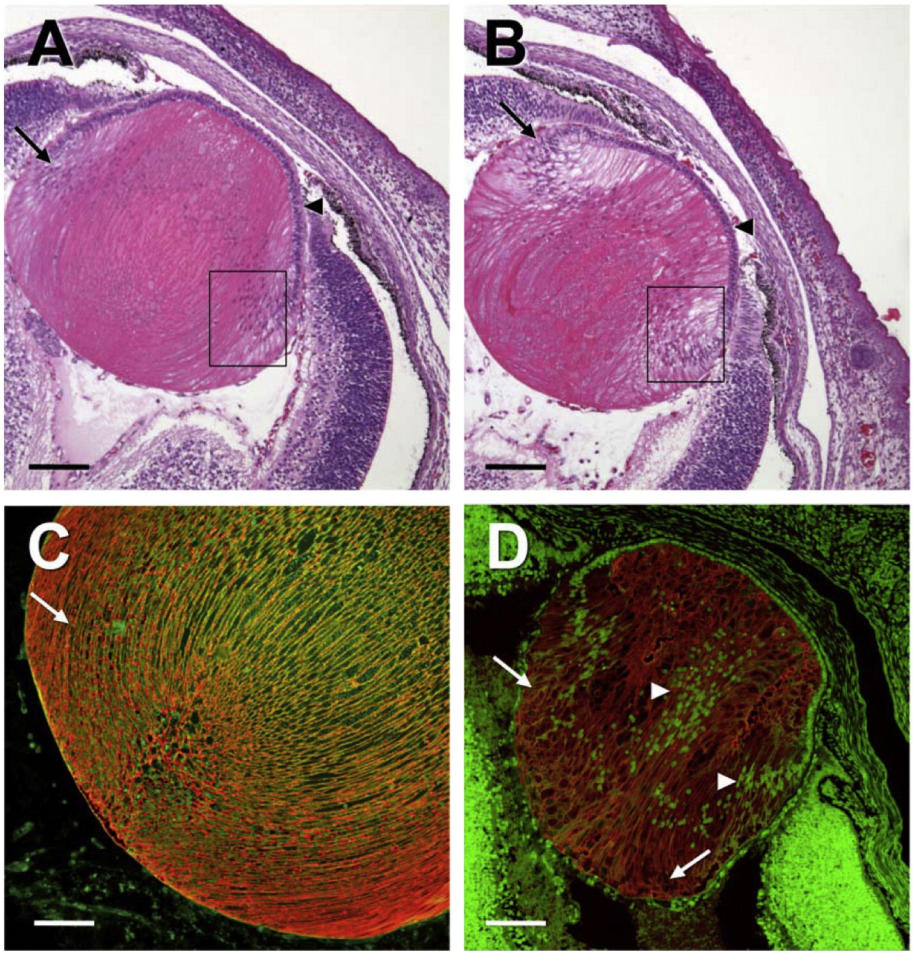

Fig. 1. Morphology of lenses from embryonic and newborn mice. Wild type and αA-R49C knock-in mutant mouse eyes were analyzed by hematoxylin/eosin staining (A, B) and aquaporin-0 immunofluorescence (C, D). Eyes from embryonic day 17 (E17) mice showed normal lens morphology (A), whereas those from αA-R49C homozygous mutants (B) have an aberrant morphology and uneven staining of cortical fiber cells (box), and increased vacuole formation in the fiber cells of the equatorial (arrows) and anterior region (arrowheads) of the lens. C) Immunofluorescence of aquaporin-0 expression in lens fiber cells of Day 0 (P0) mouse lenses. Note the regular morphology of fiber cells in the nuclear and posterior regions. (D) αA-R49C homozygous knock-in mutant mouse lenses showed irregularly shaped fiber cell morphology and a disrupted alignment of lens fiber cells. The nuclei (green) appear to accumulate in the posterior and nuclear regions in the homozygous mutant lenses (arrowheads). Red = Aquaporin-0 immunofluorescence. Green = DRAQ5. Scale bar = 150 μm (A, B, D), 100 μm (C).

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