Unfolded protein response activation in cataracts

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PURPOSE: To analyze the expression of 78 kDa glucose-regulated protein (GRP78) and activating transcription factor 6 (ATF6), 2 factors in the unfolded protein response (UPR), in age-related and diabetes-associated cataract.

SETTING: Universidad Autónoma de Aguascalientes, Aguascalientes, México.

DESIGN: Experimental study.

METHODS: The qualitative and quantitative expression of GRP78 and ATF6 were measured in surgical samples from 11 senile cataracts, 9 diabetic-associated cataracts, and 3 normal lenses. Both proteins were detected by immunofluorescence and immunogold-conjugated antibodies. Quantitative morphometry was used to analyze the differences in GRP78 and ATF6 between samples. The Mann-Whitney test was used for statistical analysis.

RESULTS: Scanning electron microscopy showed the characteristic organization of fibers in normal lenses with regular alignment and interdigitation between them. On the other hand, lenses from eyes with senile or diabetic cataract showed the same pattern of misalignment and disorganization of the fibers. Both proteins were detected through immunofluorescence in senile and diabetic cataracts, but not in normal lenses. Immunogold-conjugated antibodies and transmission electron microscopy showed that GRP78 and ATF6 grains were 30% higher and 35% higher, respectively, in diabetic cataracts than in senile cataracts ($P<.05$).

CONCLUSIONS: These data show for the first time in humans that GRP78 and ATF6 are present in lens fibers of senile cataracts and diabetic cataracts, establishing that the UPR may be important in the process of cataractogenesis.

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Cataract is defined as any opacification of the lens that causes symptoms, including decreased visual acuity, decreased color perception, decreased contrast sensitivity, and glare disability, which eventually result in blindness.[1] According to the World Health Organization, 25 million people are affected with cataract, which is also the leading cause of blindness worldwide. Age-related cataracts are responsible for nearly one half of all blindness worldwide.[2,3] Cataract is also a multifactorial disease that has been associated with systemic diseases such as diabetes, environmental factors, genetics, and other factors such as smoking[4]; however, the physiopathology remains unknown.[5]

Recently, researchers have sought to determine the role of progressive oxidative damage, protein aggregation, and glucose levels. The prevalence of cataract is 4 to 5 times higher in diabetic patients than in normal controls; high levels of glucose in the aqueous humor produce glycosylation of crystalline proteins, a process that results in the formation of advanced glycation end products and superoxide radicals. In diabetic lenses, there is an increase in the activity of aldose reductase, with the subsequent reduction of glucose to sorbitol. The increased levels of sorbitol in lens epithelial cells (LECs) cause major osmotic changes[5] that may induce stress in the endoplasmic reticulum. This stress in the endoplasmic

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reticulum could start a response to malformed proteins called the unfolded protein response (UPR).[6]

The UPR is essential to maintain cellular homeostasis,[7] and the basic components of this response are the transducers transmembrane protein kinase–endoribonuclease (IRE1), activating transcription factor 6 (ATF6), and double-stranded RNA activated protein kinase kinase-like endoplasmic reticulum (PERK), as well as the master regulator BiP/GRP78. The first transducer is the IRE1, which has 2 isoforms in mammals (IRE1-α and IRE1-β). This protein has an N terminal domain of endoplasmic reticulum stress sensor within the lumen of the organelle and a C-terminal domain with endoribonuclease activity in the cytosol. The second transducer, ATF6, is a transcription factor with an N terminal b-ZIP (basic leucine zipper) in the cytosol and a C terminal domain stress censor in the endoplasmic reticulum. In addition, PERK contains a domain stress sensor of endoplasmic reticulum and a cytosolic domain that phosphorylates eIF2α. The master controller in all this response is the endoplasmic reticulum chaperone protein, GRP78, which in nonstress conditions binds and inactivates the 3 transducers, keeping them within the endoplasmic reticulum.[8] Several stimuli within a cell, such as osmotic changes and oxidation, may converge in cellular stress. The accumulation of malformed proteins in endoplasmic reticulum formed by several stimuli may activate the UPR.

The UPR response has 2 main steps. The first begins when the accumulation of malformed proteins in endoplasmic reticulum activate 78 kDa glucose-regulated protein (GRP78), separating it from the UPR transducers. This results in the activation of the XBP1 transcription factor, which acts as a potent activator of the transcription of various genes in UPR. These genes share within their promoter a sequence consensus known as the endoplasmic reticulum stress response element.[9–11] All this leads to the transcription of chaperones and proteins whose function is essential to maintain the homeostasis of endoplasmic reticulum. The aim of this study was to analyze the expression of GRP78 and ATF6 in senile cataracts and diabetic cataracts.

MATERIALS AND METHODS

This prospective observational comparative trial included tissue samples of lenses obtained from healthy donors postmortem as well as lenses from patients with senile or diabetes-associated cataract who had extracapsular cataract extraction (ECCE) at the Department of Anterior Segment, Inova Vision Quirurgica. The study was approved by the Institutional Bioethics Committee, Autonomous University of Aguascalientes (CIB-UAA-02). Lenses were obtained from diabetic patients and nondiabetic patients who met inclusion and exclusion criteria. All patients provided written consent after receiving a detailed explanation of the procedure and the possible risks. The normal lenses were obtained postmortem from healthy donors through the Eye Bank of Aguascalientes and were used with the relatives' permission. Table 1 shows the epidemiological data for the surgical samples.

Tissue Processing

After ECCE, the cataract nucleus was divided into quadrants; each one was placed immediately in paraformaldehyde 4.0% and glutaraldehyde 3.0% solution in phosphate-buffered saline (PBS). Analysis was performed using 2 techniques; that is, immunofluorescence in paraffin sections (sensitivity in microns) and immunogold conjugation through inclusion of samples in acrylic resin (LR-White, London Resin Products) (sensitivity in nanometers)[12] to localize and quantify the presence of GRP78 and ATF6 in the samples.[13]

Immersion of Sample in Paraffin

Once the lens tissue was fixed, it was washed with water for 30 minutes and processed in histoquinet. The first drying step was performed with ascending alcohol concentrations, with each step lasting approximately 1 hour. Next, the xylene was clarified in 2 steps, each lasting 1 hour. Finally, the tissue was blocked by embedding it in paraffin in 2 steps of 2 hours each. The slides were prepared with a microtome. They were 5 µm thick and mounted on slides pretreated with 3-aminopropyltriethoxysilane (Sigma-Aldrich Co.)

Paraffin Slides Immunofluorescence

The paraffin was removed from slides by placing them in an oven at 56°C for approximately 2 hours and then in a xylene concentrate in a series of ethyl alcohol concentrations ranging from 70% to 100%. They were rinsed immediately in

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