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Aquaporin 5 knockout mouse lens develops hyperglycemic cataract

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ABSTRACT

The scope of this investigation was to understand the role of aquaporin 5 (AQP5) for maintaining lens transparency and homeostasis. Studies were conducted using lenses of wild-type (WT) and AQP5 knockout (AQP5-KO) mice. Immunofluorescent staining verified AQP5 expression in WT lens sections and lack of expression in the knockout. *In vivo* and *ex vivo*, AQP5-KO lenses resembled WT lenses in morphology and transparency. Therefore, we subjected the lenses *ex vivo* under normal (5.6 mM glucose) and hyperglycemic (55.6 mM glucose) conditions to test for cataract formation. Twenty-four hours after incubation in hyperglycemic culture medium, AQP5-KO lenses showed mild opacification which was accelerated several fold at 48 h; in contrast, WT lenses remained clear even after 48 h of hyperglycemic treatment. AQP5-KO lenses displayed osmotic swelling due to increase in water content. Cellular contents began to leak into the culture medium after 48 h. We reason that water influx through glucose transporters and glucose cotransporters into the cells could mainly be responsible for creating hyperglycemic osmotic swelling; absence of AQP5 in fiber cells appears to cause lack of required water efflux, challenging cell volume regulation and adding to osmotic swelling. This study reveals that AQP5 could play a critical role in lens microcirculation for maintaining transparency and homeostasis, especially by providing protection under stressful conditions. To the best of our knowledge, this is the first report providing evidence that AQP5 facilitates maintenance of lens transparency and homeostasis by regulating osmotic swelling caused by glucose transporters and cotransporters under hyperglycemic stressful conditions.

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1. Introduction

Cataract or lens opacity is the leading cause of visual impairment throughout the world. Over 24.4 million Americans age 40 and above are affected by cataract and more than 50% at age 80 suffer from cataract. Diabetes mellitus affects more than 285 million people worldwide [1] and is a major risk factor for cataract [2]. Onset of cataract is ~20 years earlier in diabetic patients than in non-diabetics [3]. In diabetes, high blood sugar or hyperglycemia results in ocular lens swelling leading to visual impairment. The mechanism of lens swelling has not been addressed satisfactorily thus far.

The lens of adult mammalian eye is devoid of vasculature to remain transparent for focusing objects. It consists of an epithelial cell monolayer (Fig. 1A) that extends from the anterior pole to the equatorial surface. Multilayered fiber cells form bulk of the lens, the oldest cells being deep in the interior. Secondary fiber cells differentiate from the equatorial epithelial cells and cover the older fiber cells, the youngest being at the outermost layer. To meet metabolic demands, the avascular lens tissue is postulated to have unique microcirculatory mechanisms, based on the asymmetrically distributed ion pumps, transporters, water channels and gap junction channels [4,5].

Aquaporin (AQP) water channels allow the passage of water and/or small neutral solutes across cell membranes based on osmotic gradient, thus taking part in lens microcirculation. Mutations or lack of expression of aquaporins in mammals cause pathophysiological conditions indicating their important role/s in cellular water homeostasis. Until recently, it was thought each type of lens cell expresses exclusively one specific member of the aquaporin family i.e., AQP0 in the fiber cells and AQP1 in the epithelial cells. The spatial expression of a third member of the aquaporin family, namely AQP5, in both epithelial and fiber cells was demonstrated lately [6,7] even though its presence was identified earlier by RT-PCR [8] and mass spectrometry [9]. In lens, anterior epithelial cells express AQP1 and AQP5 [10]. AQP1 functions as a water channel in the epithelial cells [11–13]. Knockout of AQP1 caused lens cataract only under stressful conditions [13]. Lens fiber cells express AQP0 and AQP5. AQP0 provides water permeability [11,14], and cell-to-cell adhesion [15,16]. Mutations as well as knockout of AQP0 caused lens cataract.

AQP5 is expressed in several secretory tissues, retina, cornea, and lens. AQP5-KO mouse model studies have corroborated the role of AQP5 in salivary secretion [17,18] and corneal thickness [19]. Phosphorylation of AQP5 results in internalization of the protein

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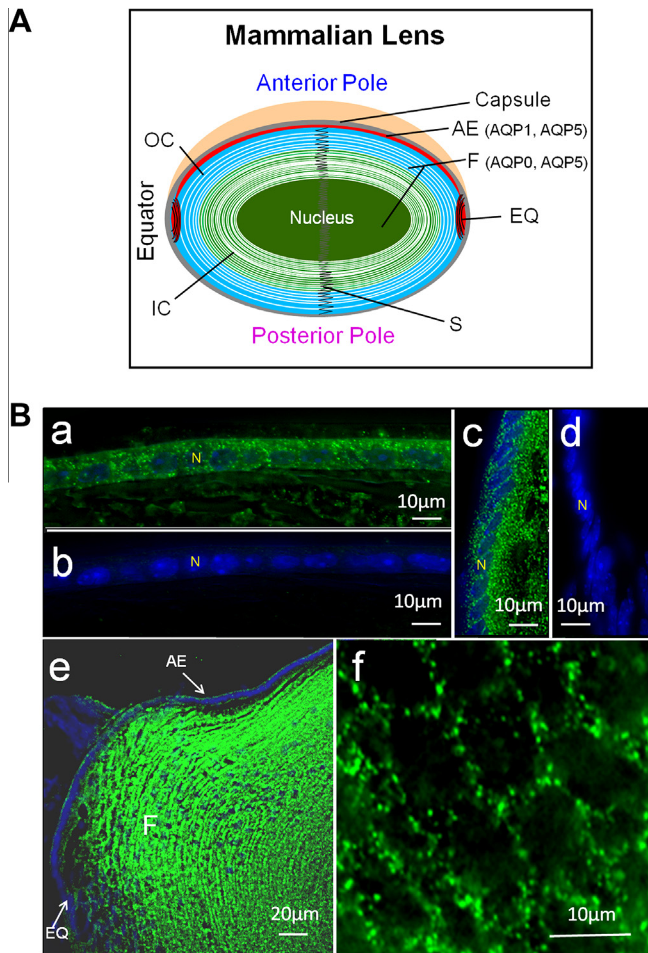

Fig. 1. (A) Schematic representation of the expression patterns of AQP0, AQP1 and AQP5 in adult mouse lens. (B). Immunostaining using anti-AQP5 antibody in lens cryosections. (a) WT epithelial cells; (b) AQP5-KO epithelial cells; (c) WT lens equatorial region showing AQP5 in epithelial and differentiating fiber cells; (d) AQP5-KO equatorial epithelial cells; (e) Sagittal section, WT lens epithelial and fiber cells showing immunoreactivity to anti-AQP5 antibody and (f) Cross-sectional view of WT cortex fiber cells showing AQP5 expression. (a–f) FITC-conjugated secondary antibody; green, antibody binding; blue, nuclear staining by DAPI. White arrows – antibody binding. AE, anterior epithelial cells; EQ, equatorial epithelial cells; F, fiber cells; IC- Inner cortex; N-nucleus; OC- outer cortex; S- suture. (For interpretation of color in Fig. (1), the reader is referred to the web version of this article.)

from plasma membrane [6]. Even though the spatial distribution of AQP5 in lens has been studied, there is no research yet to find out whether it has any role in maintaining lens transparency and homeostasis. The aim of the current investigation was to explore the involvement of AQP5 in lens transparency and homeostasis.

In this study, we tested WT and AQP5-KO lenses under hyperglycemic condition *ex vivo* and demonstrated for the first time that presence of AQP5 is critical for maintaining lenticular osmotic balance as was evident from cataractogenesis of the experimental lenses in contrast to control. Moreover, we provide possible answers for: Why and how does swelling occur in lens under hyperglycemic condition? This study opens up a realm of future explorations with the potential for developing therapeutic strategies for senile and diabetic cataracts.

2. Materials and methods

2.1. Animals, lens isolation and immunofluorescent staining

Wild type (WT; C57BL/6J: Jackson Laboratory) and AQP5 knockout (AQP5-KO) mice (provided by Dr. A.G. Menon, Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati Medical Center, Cincinnati, OH [18]) were used. All procedures were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by Stony Brook University Animal Care and Use Committee. Lenses were dissected out from eyes under a light microscope without damaging lens capsules. Immunofluorescent staining was performed as described previously [6].

2.2. Ex vivo model of cataract formation

Hyperglycemia-induced *ex vivo* cataractogenesis was studied as previously described [13,20]. Lenses from WT and AQP5-KO mice were dissected out aseptically, rinsed in saline and transferred to 12-well culture plates containing culture medium supplemented with 5.6 mM (normal, control) or 55.6 mM (hyperglycemic) glucose. Incubations were done at 37 °C in a humidified atmosphere with 5% CO_2. Lens opacity was quantified from the images taken using a digital camera-fitted stereo-zoom microscope [12].

2.3. Lens water content, transparency and focusing ability

Water content of four month-old WT and AQP5-KO mouse lenses was quantified by measuring wet and dry weights after 60 h of hyperglycemic stress. Lenses (8 each from WT and AQP5-KO mice) were weighed for wet weight, dried in a vacuum oven (98 °C, 24 h) and weighed again for dry weight. Percentage of water content was calculated [21]: (wet weight − dry weight) × 100/wet weight), expressed as mean ± SD and analyzed using Paired *t*-test.

Lens transparency was quantified as described by Varadaraj et al. [12]. In brief, lenses treated with normal or hyperglycemic medium for 48 h were photographed digitally and analyzed (SigmaScan Pro, Version 5.0). The transparency index of each lens was calculated by dividing total intensity by number of pixels and was high in less-transparent lenses.

Focusing ability was assessed qualitatively using grid images of normal glucose (5.6 mM)-treated and hyperglycemic (55.6 mM glucose) WT and AQP5-KO lenses after 24 h. Imaging was performed under dark field [21].

3. Results and discussion

Immunofluorescent staining of WT and AQP5-KO mouse lens cryosections (Fig. 1B) verified our previous observations [6]. AQP5 expression in the WT lens epithelial cells (Fig. 1B a) was corroborated by negative immunostaining of AQP5-KO lens (Fig. 1B b). WT lens equatorial region showed positive staining (Fig. 1B c) in contrast to AQP5-KO (Fig. 1B d). Overall, AQP5 was expressed in the epithelial and fiber cells (Fig. 1B e). Enlarged image of cortical fiber cells demonstrated AQP5 in the plasma membrane (Fig. 1B f). In epithelial cells, expression of AQP5 was several fold less than AQP1 [6].

Expression of a second aquaporin in fiber cells (apart from AQP0) and epithelial cells (apart from AQP1) prompted us to explore its functional significance. Since AQP5-KO mouse lenses appeared as normal as those of WT, we followed hyperglycemia-induced *ex vivo* cataractogenesis model to investigate whether AQP5 plays a role in lens microcirculation. The glucose concentration selected for hyperglycemia induction was 55.6 mM, as observed in diabetic cataracts *in vivo* [22] and had been used customarily to induce cataract [13] as well as to test the cataractogenic effects of drugs [23]. We tested two concentrations for WT and AQP5-KO lenses; 5.56 mM which is equivalent to the normal glucose level, and 55.6 mM hyperglycemic level. After 24 h of incubation, only high glucose-treated lenses of AQP5-KO mouse

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