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Myo/Nog cells are present in the ciliary processes, on the zonule of Zinn and posterior capsule of the lens following cataract surgery

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ABSTRACT

Myo/Nog cells, named for their expression of MyoD and noggin, enter the eye during early stages of embryonic development. Their release of noggin is critical for normal morphogenesis of the lens and retina. Myo/Nog cells are also present in adult eyes. Single nucleated skeletal muscle cells designated as myofibroblasts arise from Myo/Nog cells in cultures of lens tissue. In this report we document the presence of Myo/Nog cells in the lens, ciliary body and on the zonule of Zinn in mice, rabbits and humans. Myo/Nog cells were rare in all three structures. Their prevalence increased in the lens and ciliary body of rabbits 24 h following cataract surgery. Rabbits developed posterior capsule opacification (PCO) within one month of surgery. The number of Myo/Nog cells continued to be elevated in the lens and ciliary body. Myo/Nog cells containing alpha smooth muscle actin and striated muscle myosin were present on the posterior capsule and overlaid deformations in the capsule. Myo/Nog cells also were present on the zonule fibers and external surface of the posterior capsule. These findings suggest that Myo/Nog contribute to PCO and may use the zonule fibers to migrate between the ciliary processes and lens.

Myo/Nog cells constitute a novel lineage with multiple functions in the embryo and adult. These cells were originally identified in the epiblast of blastocyst stage chick embryo by their expression of messenger RNA (mRNA) for the skeletal muscle specific transcription factor MyoD, the bone morphogenetic protein (BMP) inhibitor noggin and labeling with the G8 monoclonal antibody (mAb) (Gerhart et al., 2000, 2006, 2009; Strony et al., 2005). During early stages of morphogenesis, Myo/Nog cells are integrated from the epiblast into tissues and organs derived from all three germ layers, including the eyes (Gerhart et al., 2006, 2007, 2009). Myo/Nog cells continue to express MyoD and noggin regardless of their environment (Gerhart et al., 2001, 2007, 2009). Elimination of Myo/Nog cells in the epiblast results in hyperactive BMP signaling, malformations of the nervous system, body wall and face, an absence of skeletal muscle, and ectopic cardiac muscle (Gerhart et al., 2006, 2009, 2011). Additionally, embryos depleted of Myo/Nog cells develop eye defects ranging from anophthalmia to dysgenesis of the lens and retina (Gerhart et al., 2006, 2009).

Myo/Nog cells are also present in adult mammalian tissues (Brandli et al., 2017; Bravo-Nuevo et al., 2016; Gerhart et al., 2012, 2014, 2017). A neuroprotective function of Myo/Nog cells was revealed in the retina of neonatal mice exposed to hyperoxia and adult rats with light induced retinopathy (Brandli et al., 2017; Bravo-Nuevo et al., 2016). Anterior lens tissue removed from patients during cataract surgery contains Myo/Nog cells that surround wounds in the epithelium and wrinkles in the capsule (Gerhart et al., 2014, 2017). Depletion of Myo/Nog cells in human lens explant cultures prevents the emergence of single nucleated, skeletal muscle cells designated as myofibroblasts (Gerhart et al., 2014, 2017).

In this report, we document the presence of Myo/Nog cells in the mouse, rabbit and human lens, ciliary processes and on the zonule of Zinn, a circumferential system of fibers that connect the ciliary body and lens (Zinn, 1755). The zonule of Zinn, also known as the ciliary zonule, is responsible for lens centration and transmitting the force of contraction from the ciliary body muscle to the lens for accommodation. Zonule fibers, consisting of bundles of microfibrils composed of fibrillins 1 and 2, microfibrillar-associated protein 2, collagens,

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proteoglycans and other proteins, insert into the lens in the zone of proliferating epithelial cells (Cain et al., 2006; Collin et al., 2015; De Maria et al., 2017; Hubmacher et al., 2014; Inoue et al., 2014; Shi et al., 2013).

The following methods were employed in this study. The globes of two C57 black 6 mice were enucleated, frozen in dry ice-cooled propane, fixed in 97% methanol and 3% acetic acid, and stored at −80° for 48 h (Sun et al., 2015). Eyes were warmed slowly, transferred to 100% ethanol and then xylene, and embedded in paraffin. Tissue was sectioned at 4 μm. Three human eyes from three donors were procured 3–13 h postmortem through the National Disease Research Interchange (Philadelphia, PA). The eyes were placed in a modified Davidson's fixative containing 14% ethyl alcohol, 14% formalin and 6.25% glacial acetic acid (Excalibur Pathology, Inc., Oklahoma City, OK). Anterior segments were embedded in paraffin and sectioned at 10 μm.

Anterior segments also were obtained from two female New Zealand white rabbits weighing between 2.8 and 3.2 kg. Additional rabbits underwent cataract surgery as described previously (Bozukova et al., 2015; Kramer et al., 2015; Li et al., 2016). Briefly, the pupil was dilated, a 3.0-mm corneal-scleral incision was made and a 5.0-mm capsulorhexis was performed after injection of viscoelastic material. The residual cortex was removed with the irrigation/aspiration handpiece following hydrodissection and phacoemulsification (INFINITI® Vision System, Alcon, Fort Worth, TX). Viscoelastic material was used to expand the capsular bag and the intraocular lens (IOL) (SA60AT; single-piece hydrophobic acrylic; AcrySof lens manufactured by Alcon, Fort Worth, TX) was then inserted into the capsular bag using the recommended injection system (Monarch III injector with “C” cartridges, Alcon). The wound was closed with 10.0 monofilament nylon suture after removal of viscoelastic material and injection of balanced salt solution.

Four and 13 globes were enucleated at 24 h and four weeks following surgery, respectively. Posterior capsule opacification (PCO) was scored on day-30 by slit lamp examination as described previously (Nishi and Nishi, 1999; Werner et al., 2001). Eyes were placed in 10% neutral buffered formalin for a minimum of 24 h and bisected coronally just anterior to the equator. Tissue was embedded in paraffin and sectioned at 10 μm.

Paraffin embedded tissue sections from all three species were stained with hematoxylin and eosin (H&E) or labeled with fluorescent antibodies as described previously (Gerhart et al., 2000, 2001, 2006). Sections were permeabilized with 0.1% Triton X-100, treated with 0.1% sodium citrate and incubated with the G8 IgM mouse mAb diluted 1:40 (Gerhart et al., 2001), G8 and a goat polyclonal antiserum to noggin diluted 1:100 (AF719; R&D Systems, Minneapolis, MN), or combinations of G8 or noggin antibodies and mAbs to alpha smooth muscle actin (α-SMA) directly conjugated with fluorescein diluted 1:250 (Sigma-Aldrich, St. Louis, MO) or vimentin diluted 1:400 (AMD-17b mAb, Developmental Studies Hybridoma Bank, Iowa City, IA). Sections also were double labeled with the MF20 antibody to striated muscle myosin heavy chain (Bader et al., 1982) diluted 1:40 (Developmental Studies Hybridoma Bank) and an antibody to MyoD1 diluted 1:100 (Vector Laboratories, Inc., Burlimghame, CA). The 11C1.3 antibody to fibrillin 1 (Ashworth, J, Br J Ophthalmology, 2000, 84, 1312) diluted 1:250 (Fisher/Invitrogen, Philadelphia, PA) was used to stain the zonule fibers in rabbit and human tissue sections. The polyclonal antibody to fibrillin 2 (ThermoFisher Scientific, Waltham, MA) was applied to tissue sections from the mouse.

Primary antibodies were visualized with affinity purified, F(ab')2 goat anti-mouse IgM μ chain conjugated with rhodamine, donkey anti-goat IgG conjugated with Dylight 488, goat anti-mouse IgG2b conjugated with rhodamine, goat anti-mouse IgG1 and goat-anti-mouse IgG conjugated with fluorescein (Jackson ImmunoResearch, West Grove, PA). Secondary antibodies were diluted 1:400. Nuclei were labeled with Hoechst dye. Background fluorescence was assessed by incubating tissue sections with secondary antibodies alone. Lens fiber cells had a stronger, homogeneous, fluorescent hue than the rest of the tissue (not shown). Occasionally, a low level of punctate fluorescence was observed within the lens capsule.

Antibody labeling was analyzed with the Nikon Eclipse E800 epifluorescence microscope equipped with the Evolution QE Optronics video camera and Image Pro Plus image analysis software program (Media Cybernetics, Rockville, MD), and the Nikon Eclipse 90i with Roper camera and Nikon Elements Advanced Research software. Figures were annotated and adjusted for brightness and contrast with Adobe Photoshop CC 2014.

Means and standard deviations were calculated for cells labeled with antibodies to G8 and noggin, G8 or noggin and α-SMA, and sarcomeric myosin heavy chain and MyoD1 in the lens and ciliary body. The numbers of cells were compared between normal eyes and rabbit eyes 24 h and four weeks after cataract surgery by the *t*-test.

The length and width of the ciliary processes of normal rabbit eyes and those that had undergone cataract surgery were measured in H&E stained sections that were photographed with a Nikon Eclipse Ti-inverted microscope with a Nikon Ds-Qi2 camera. The mean ± standard deviation was calculated for the length and width of 11 ciliary processes of two normal rabbit eyes, and 21 and 24 ciliary processes from three eyes 24 h and 30 days after cataract surgery, respectively. The t-test was used to compare the ciliary processes of normal and post-operative eyes.

Rabbits and mice were treated in accordance with guidelines set forth by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the IACUC committees of the University of California, Davis and University of Utah. Human tissue procurement by the NDRI followed The Code of Ethics of the World Medical Association (Declaration of Helsinki). Informed consent was obtained for all patients.

We previously reported that Myo/Nog cells expressing MyoD mRNA, noggin and the G8 antigen were present in the ciliary body and lens of the human eye (Gerhart et al., 2014). In this report, we demonstrate G8-positive (+)/noggin + Myo/Nog cells in the anterior segment of mouse and rabbit eyes (Fig. 1). Myo/Nog cells were found in the equatorial and bow regions of the lens in mice (3.4 ± 1.1/section, n = 5 sections from 2 eyes), rabbits (2.6 ± 0.8 cells/section, n = 21 sections/2 eyes) and humans (4.9 ± 3.6 cells/section, n = 36 sections/3 eyes) (Fig. 1B, C, G, H, L and M). The ciliary processes also contained low numbers of Myo/Nog cells within the stroma in all three species (mice: 5.4 ± 0.9 cells/section, n = 5 sections/2 eyes; rabbits: 2.5 ± 1.4 cells/section, n = 11 sections/2 eyes; humans: 2.6 ± 1.9 cells/section, n = 36 sections/3 eyes) (Fig. 1D, I and N). In the rabbit, these cells were occasionally found among the ciliary epithelial cells (Fig. 1I). A few Myo/Nog cells (1–4) were associated with the zonule in mice, rabbits and humans (Fig. 1E, J and O); however, cells were not observed on most zonule fibers. In all three structures, some cells appeared to contain cytoplasmic G8 mAb staining (Fig. 1). This finding is consistent with our previous study demonstrating that Myo/Nog cells in human lens explant cultures internalize G8 antibody/antigen complexes into acidic compartments of the cytoplasm (Gerhart et al., 2017).

The anterior segments of rabbits that had undergone cataract surgery were examined for Myo/Nog cells. A day after surgery, G8+ cells were visible in the equatorial region of the lens (Fig. 2B). The number of Myo/Nog cells was elevated (5 ± 0.8 cells/section, n = 8 sections from 4 eyes) compared to normal eyes (p = 0.0001). An increase in Myo/Nog cells following surgery also was observed in the ciliary processes (7 ± 5 cells/section, n = 21 sections/6 eyes, p = 0.06) in which they were present in the stroma (Fig. 2C) and associated with the epithelium (Fig. 2C–E). The width of the ciliary processes appeared enlarged (2.4 ± 1.1 cm, n = 21 processes/3 eyes) compared to eyes that had not undergone surgery (0.95 ± 0.42, n = 11 processes/2 eyes, p = 0.0002). Myo/Nog cells were associated with some but not all zonules fibers (Fig. 2F).