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Beta-1 integrin is important for the structural maintenance and homeostasis of differentiating fiber cells

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ABSTRACT

β1-Integrin is a heterodimeric transmembrane protein that has roles in both cell–extra-cellular matrix and cell–cell interactions. Conditional deletion of β1-integrin from all lens cells during embryonic development results in profound lens defects, however, it is less clear whether this reflects functions in the lens epithelium alone or whether this protein plays a role in lens fibers. Thus, a conditional approach was used to delete β1-integrin solely from the lens fiber cells. This deletion resulted in two distinct phenotypes with some lenses exhibiting cataracts while others were clear, albeit with refractive defects. Analysis of "clear" conditional knockout lenses revealed that they had profound defects in fiber cell morphology associated with the loss of the F-actin network. Physiological measurements found that the lens fiber cells had a twofold increase in gap junctional coupling, perhaps due to differential localization of connexins 46 and 50, as well as increased water permeability. This would presumably facilitate transport of ions and nutrients through the lens, and may partially explain how lenses with profound structural abnormalities can maintain transparency. In summary, β1-integrin plays a role in maintaining the cellular morphology and homeostasis of the lens fiber cells.

1. Introduction

The crystalline lens is a transparent tissue consisting of a basement membrane, the lens capsule, which surrounds two cell types, lens epithelial cells (LECs) on the anterior surface, which proliferate and differentiate into lens fiber cells (LFCs) that make up the majority of the lens (Kuszak et al., 1988; Piatigorsky, 1981). Similar to other tissues (Ingber, 2003a, b, the cells of the lens must interact with both the extracellular matrix and each other to maintain the structural integrity of the tissue, and to regulate cell function (Yamada et al., 2000). While lens cells express a wide array of cell adhesion molecules capable of mediating such interactions, relatively little is known about the function of many of these molecules in the lens and how these functions change as LECs differentiate into lens fibers.

Integrins are heterodimeric transmembrane proteins comprised of an α- and β-subunit, which play major roles in cell–ECM adhesion and cell signaling in all vertebrate tissues (Hynes, 1992). Currently, 18 alpha and 8 beta subunits are found in mammals which assemble into 24 functional heterodimers (Hynes, 2004). Each heterodimer has discrete functions resulting from both differences in their interactions with ECM components and other ligands, as well as the cell signaling pathways that they regulate (Hynes, 1987). Thus, integrins are important players in both the maintenance of tissue integrity and the regulation of cellular identity in animals.

The lens expresses a diverse complement of integrins which have been ascribed various functions in lens development and disease (Walker and Menko, 2009). Mice lacking both the α3 and

Abbreviations: LECs, lens epithelial cells; LFCs, lens fiber cells; ECM, extracellular matrix; IGF, insulin-like growth factor; OCFs, outer cortical fibers; ICFs, inner cortical fibers; F-actin, filamentous actin; cKO, conditional knockout; ILK, integrin linked kinase; G_{DF}, coupling conductance of differentiating fibers; G_{MF}, coupling conductance of mature fibers; G_m, membrane conductance; G_s, surface cell membrane conductance; R_e, extracellular resistivity; ESC, embryonic stem cells; Cx, connexin; SIM, structured illumination microscopy.

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α6 integrin genes exhibit profound defects in the lens epithelium (De Arcangelis et al., 1999), while conditional deletion of the β1-integrin gene from all lens cells after primary fiber cell elongation leads to disorganization of the lens epithelium with ectopic expression of α-smooth muscle actin at E16.5, and finally inappropriate LEC apoptosis by birth (Simirskii et al., 2007). β1-Integrins are also expressed by LFCs where they have been proposed to be crucial for fiber cell–lens capsule interactions (Bassnett et al., 1999). Notably, β1-integrins are also present on the lateral membranes of cortical fiber cells (Duncan et al., 2000b; Menko and Philip, 1995; Simirskii et al., 2007) while α6-integrin, a major partner of β1-integrin in embryonic chicken lens fibers (Menko and Philip, 1995; Walker and Menko, 1999), can co-signal with the IGF receptor and regulate fiber cell differentiation in primary cell culture (Walker et al., 2002). However, while deletion of β1-integrin from all embryonic lens cells did result in severe lens fiber cell defects and lens degeneration (Samuelsson et al., 2007; Simirskii et al., 2007), it was not possible to separate direct functions of β1-integrin in the lens fibers from indirect ones caused by loss of β1-integrin from the lens epithelium. Here, β1-integrin was solely deleted from the LFCs, while leaving lens epithelial expression intact, revealing a role for β1-integrin in lens fiber cell structure and physiology.

2. Methods

2.1. Animals

All animal experiments described here conform to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. *Itgb1*^{tm1Efu}*/J* mice, which harbor an allele of the β1 integrin gene in which exon 3 is flanked by LoxP sites (Raghavan et al., 2000), were obtained from The Jackson Laboratory (Bar Harbor, Maine) and genotyped as described (Simirskii et al., 2007). MLR39-cre mice on a FVB/N background expressing Cre recombinase in LFCs from E12.5 onward (Zhao et al., 2004) were obtained from Michael L. Robinson (Miami University, Oxford, OH) and genotyped as described (Zhao et al., 2004). *Itgb1*^{tm1Efu}*/J* mice were bred to MLR39 mice to create mice lacking β1-integrin in lens fibers (cKO) (Simirskii et al., 2013). All conditional knockout mice are mutant at the CP49 locus consistent with the genetic background of the founder strains of these mice (Alizadeh et al., 2004; Sandilands et al., 2003; Simirskii et al., 2006). The wild type mice used in these studies were of either the CP49 + C57Bl/6⟨Har⟩ strain or the CP49 – FVBN strain (designated in the figure legends) (Simirskii et al., 2006). All mice were maintained under specific pathogen free conditions at the University of Delaware animal facility under a 14/10-h light/dark cycle. Embryos were staged by designating the appearance of a vaginal plug in the dam as 0.5 days embryonic (E). All experiments were done using closely age matched experimental and control animals; the ages of the animals used in each experiment are noted in the figure legends.

2.2. Gross morphology

Eyes were enucleated, lenses dissected, and placed into pre-warmed tissue culture Medium 199, 1× (with Earle's salts and L-glutamine) (Cellgro, Mediatech, Inc., Manassas, VA). Transparency was assessed by photographing lenses using a Zeiss Stemi SV dissecting microscope fitted with a darkfield base. The refractive properties of lenses were assessed by placing them on a 200 mesh electron microscopy grid then photographing them using bright-field with a dissecting microscope, optimizing focus upon the grid, as previously described (Shiels et al., 2007).

2.3. Histology

Eyes (postnatal mice) were removed immediately after sacrifice and fixed in Pen-Fix (Richard Allan Scientific, Kalamazoo, MI) for 2 h. Samples were then transferred into 70% ethanol and paraffin embedded. Six-micron thick sections were prepared, stained by hematoxylin and eosin (H&E) and imaged on a Zeiss Axiophot fitted with a digital Nikon camera.

2.4. Immunofluorescence

Fluorescent immunolocalization on longitudinal eye sections was done as previously described (Reed et al., 2001). Briefly, eyes were removed, embedded directly in Optimum Cutting Temperature media (OCT, Tissue Tek, Torrance, CA), 16 μm thick sections created on a Leica cryostat (Leica) and mounted on slides (ColorFrost Plus, Fisher Scientific Hampton, NH). Slides were immersion fixed in 1:1 acetone–methanol at −20 °C for 15 min or 4% paraformaldehyde for 30 min depending on the antibody, and blocked in 1% BSA (Bovine Serum Albumin, Sigma Aldrich) for 1 h at room temperature. Sections were then incubated with primary antibody diluted with blocking buffer under conditions described in Table 1. Sections were washed in either 1× PBS or 1× TBS depending on antibody, and incubated with the appropriate Alexa Fluor 568 or Alexa Fluor 488 labeled secondary antibody (Invitrogen, Grand Island, NY) at a dilution of 1:200 and the DRAQ-5 nucleic acid stain (Biostatus Limited, Shepshed, Leicestershire, UK) at a dilution 1:2000 in either 1× PBS or 1× TBS as appropriate. Sections were washed again in 1× PBS or 1× TBS and then mounted in mounting media (10 ml of PBS with 100 mg of p-phenylenediamine to 90 ml of glycerol; final pH 8.0).

Equatorial sections were stained similarly, except that the sections were 20 μm thick and a 1:200 dilution of Alexa Fluor 488 labeled wheat germ agglutinin (Invitrogen, Grand Island, NY), which binds N-acetyl-D-glucosamine and sialic acids, was added to the secondary antibody solution to visualize cell membranes (Ohno et al., 1986).

2.5. F-actin visualization

For whole mount analyses, lenses were dissected from the eye, fixed for 2 h in 4% paraformaldehyde then washed in 1× PBS with 0.1% Triton X-100. Samples were stained in 1% BSA in 1× PBS with 0.25% Triton X-100, a 1:2000 dilution of DRAQ5 (Biostatus Limited, Shepshed, Leicestershire, UK), and a 1:200 dilution of Alexa Fluor 568 labeled phalloidin overnight at 4 °C. Lenses were then washed in 1× PBS with 0.1% Triton X-100 and stored in the dark at 4 °C until imaged.

Twenty micrometer thick equatorial sections were fixed in 4% paraformaldehyde for 30 min and blocked in 1% BSA for 1 h at room temperature. Sections were then incubated with Alexa Fluor 568 labeled phalloidin (Invitrogen, Grand Island, NY) at a 1:200 dilution for 1 h at room temperature and then washed in 1× PBS. Sections were counterstained in a solution containing a 1:200 dilution of Alexa Fluor 488 labeled wheat germ agglutinin (Invitrogen, Grand Island, NY) and a 1:2000 dilution of the nucleic acid stain DRAQ5 for 1 h at room temperature. Sections were washed again in 1× PBS, and then mounted.

For β1 integrin and F-actin co-staining sixteen micrometer thick longitudinal sections were fixed in 4% paraformaldehyde for 30 min and blocked in 1% BSA for 1 h at room temperature. Sections were incubated with primary β1 integrin antibody (described in Table 1). Sections were washed in 1× PBS then incubated with appropriate Alexa Fluor 488 antibody (1:200), Alexa Fluor 568