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Gene expression signatures in tree shrew choroid during lens-induced myopia and recovery

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ABSTRACT

Gene expression in tree shrew choroid was examined during the development of minus-lens induced myopia (LIM, a GO condition), after completion of minus-lens compensation (a STAY condition), and early in recovery (REC) from induced myopia (a STOP condition). Five groups of tree shrews ($n = 7$ per group) were used. Starting 24 days after normal eye-opening (days of visual experience [DVE]), one minus-lens group wore a monocular −5 D lens for 2 days (LIM-2), another minus-lens group achieved stable lens compensation while wearing a monocular −5 D lens for 11 days (LIM-11); a recovery group also wore a −5 D lens for 11 days and then received 2 days of recovery starting at 35 DVE (REC-2). Two age-matched normal groups were examined at 26 DVE and 37 DVE. Quantitative PCR was used to measure the relative differences in mRNA levels in the choroid for 77 candidate genes that were selected based on previous studies or because a whole-transcriptome analysis suggested their expression would change during myopia development or recovery. Small myopic changes were observed in the treated eyes of the LIM-2 group (−1.0 ± 0.2 D; mean ± SEM) indicating eyes were early in the process of developing LIM. The LIM-11 group exhibited complete refractive compensation (−5.1 ± 0.2 D) that was stable for five days. The REC-2 group recovered by 1.3 ± 0.3 D from full refractive compensation. Sixty genes showed significant mRNA expression differences during normal development, LIM, or REC conditions. In LIM-2 choroid (GO), 18 genes were significantly down-regulated in the treated eyes relative to the fellow control eyes and 10 genes were significantly up-regulated. In LIM-11 choroid (STAY), 10 genes were significantly down-regulated and 12 genes were significantly up-regulated. Expression patterns in GO and STAY were similar, but not identical. All genes that showed differential expression in GO and STAY were regulated in the same direction in both conditions. In REC-2 choroid (STOP), 4 genes were significantly down-regulated and 18 genes were significantly up-regulated. Thirteen genes showed bi-directional regulation in GO vs. STOP. The pattern of differential gene expression in STOP was very different from that in GO or in STAY. Significant regulation was observed in genes involved in signaling as well as extracellular matrix turnover. These data support an active role for the choroid in the signaling cascade from retina to sclera. Distinctly different treated eye vs. control eye mRNA signatures are present in the choroid in the GO, STAY, and STOP conditions. The STAY signature, present after full compensation has occurred and the GO visual stimulus is no longer present, may participate in maintaining an elongated globe. The 13 genes with bi-directional expression differences in GO and STOP responded in a sign of defocus-dependent manner. Taken together, these data further suggest that a network of choroidal gene expression changes generate the signal that alters scleral fibroblast gene expression and axial elongation rate.

1. Introduction

Studies of postnatal refractive development in both children and in animal models have found that there is a visually-guided emmetropization mechanism that uses refractive error to guide the growth of the eye so that the axial length eventually matches the location of the focal plane, producing visual images that are focused on the photoreceptors (emmetropia) (Mutti et al., 2005; Norton, 1999; Wallman and Winawer, 2004). In animal models,

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the emmetropization mechanism can be manipulated with lenses, held in front of one (or both) eyes in a goggle frame. Minus-power (negative) lenses shift the focal plane away from the cornea, making the eye hyperopic. This produces retinal GO signals that cause an increase in the axial (vitreous chamber) elongation rate, moving the retina to the shifted focal plane and restoring emmetropia while the lens is in place (Irving et al., 1991, 1995; Norton et al., 2010). In tree shrews, the eye remains elongated as long as the lens is left in place, evidently because some form of STAY signal reaches the sclera (Norton et al., 2010). When the lens is removed, the increased axial length causes the eye to experience lens-induced myopia (LIM). The retina then generates STOP signals that, in juvenile animals where the eyes are still growing, rapidly slow the axial elongation rate to below normal, producing recovery (REC) from the induced myopia (Norton et al., 2010).

Although the emmetropization mechanism performs more effectively in a fully intact animal, eyes can still respond to myopiagenic stimuli if the optic nerve is cut (Troilo, 1990; Wildsoet and McFadden, 2010) or output is functionally blocked with tetrodotoxin (Norton et al., 1994). In addition, covering only half of the visual field with a minus lens produces elongation and myopia only in the affected visual field (Diether and Schaeffel, 1997; Norton and Siegwart, 1991; Smith et al., 2010). Thus, there is a direct, spatially-localized signaling cascade from the retina to the sclera that must pass through the retinal pigment epithelium (RPE) and choroid. The choroid, in addition to being the vascular supply to the photoreceptors, RPE, and sclera (Birol et al., 2007; Lutty et al., 2010; Oyster, 1999), plays an important role in the emmetropization mechanism (Nickla and Wallman, 2010; Summers, 2013; Wallman and Winawer, 2004) as a way-station in the signaling cascade that conveys the GO, STAY, and STOP signals to the sclera. The extent to which some signaling molecules generated by the retina or RPE may simply pass through the choroid on the way to the sclera and other, new, signaling molecules are generated by the choroid is not known. Examining gene expression changes in the choroid alone, relative to changes in the retina and RPE, may help clarify the situation.

In species such as chick, the choroid is thick because there are no blood vessels on the vitreal side of the retina and the choroid is the sole retinal vascular supply. Chick choroid displays rapid changes within a few hours after the onset of LIM and REC, thinning during myopia development and thickening during recovery (Wallman et al., 1995). Previous studies of chick choroid have reported changes in gene expression during the development of LIM, form deprivation-induced myopia (FDM), and recovery from FDM, suggesting the choroid plays an active role producing new signaling molecules (Mertz and Wallman, 2000; Nickla et al., 2009; Nickla and Wildsoet, 2004; Nickla et al., 2006; Rada et al., 2012; Rada et al., 2001; Rada and Wiechmann, 2009; Rada et al., 2010; Simon et al., 2004). In mammals (guinea pig, tree shrew, marmoset, and macaque), the choroid is not as thick, proportionally, as in chicks and seems to undergo smaller changes in thickness during myopia development and recovery (Gentle and McBrien, 1999; Howlett and McFadden, 2009; Hung et al., 2000; Siegwart and Norton, 1998; Troilo et al., 2000).

A previous study in the marmoset examined gene expression in the combined RPE and choroid (Shelton et al., 2008). While a number of changes were found, it is not possible to know if the changes occurred in the RPE or choroid. In tree shrews (mammals closely related to primates; Luckett, 1980) and guinea pigs, studies have examined changes of a few genes and proteins in the choroid in form-deprived animals (Cui et al., 2010; Jobling et al., 2009; Liu et al., 2007; McBrien et al., 2009). In the present study, we examined, in the choroid, changes in gene expression of a large sample of genes in animals treated with a minus lens.

During the development of LIM in tree shrews, the GO signals from the choroid produce remodeling of the scleral extracellular matrix that increases the viscoelasticity of the sclera (measured as increased creep rate), allowing normal intraocular pressure to expand the globe (Phillips et al., 2000; Siegwart and Norton, 1999). With continued lens wear, STAY signals from the choroid must be present because tree shrew eyes remain in an elongated state until minus lens-wear is discontinued (Norton et al., 2010). During REC, the STOP signals from the choroid cause a rapid reduction the creep rate that slows the axial elongation rate (Siegwart and Norton, 1999).

The time-course of remodeling in the tree shrew sclera is rapid, but not instantaneous. After one day of LIM, there is little alteration of mRNA levels (Gao et al., 2011). After 2 days of LIM, scleral gene expression is altered; a scleral GO signature is found (Guo et al., 2013) and creep rate is elevated (Siegwart and Norton, 1999). Very similar, somewhat stronger gene expression changes are found after four days of LIM (Frost and Norton, 2012; Guo et al., 2013) and scleral creep rate reaches a peak at this time (Siegwart and Norton, 1999). After 11 days of LIM, the eyes have fully compensated for the minus lens but scleral viscoelasticity remains slightly elevated (Siegwart and Norton, 1999) and some gene expression differences remain in the sclera (Guo, personal communication, 2013) suggesting the presence of STAY signals in the choroid at this time point. During recovery, the scleral gene expression after one day is little changed (Guo et al., 2012); after two days of recovery, a scleral STOP remodeling response has developed (Guo, personal communication, 2013) and scleral creep rate has dropped to, or below, normal (Siegwart and Norton, 1999). Based on this time-course, it is expected that GO and STOP signals should be detectable in the choroid after two days of LIM and after two days of REC respectively.

The goal of the present study was to examine alterations in gene expression in the choroid, measured as alterations in mRNA levels, after two days of LIM (GO), 11 days of LIM (STAY), and after two days of REC (STOP). Although changes in levels of proteins or other molecules presumably are key to actually transmitting signals from choroid to sclera, it has been found that changes in mRNA can help to identify the responses of the cells in tissues and are useful in identifying pathways of interest (Gao et al., 2011; Guo et al., 2013; He et al., 2011; Schippert et al., 2006; Shelton et al., 2008; Siegwart and Norton, 2005; Stone et al., 2011; Zhang et al., 2012). Based on these previous studies, our hypothesis was not only that many of the genes examined would show changes in mRNA expression but also that the pattern of differential gene expression would differ in the GO, STAY, and STOP conditions.

2. Materials and methods

2.1. Experimental groups

The juvenile tree shrews (*Tupaia glis belangeri*) used in this study were produced in our breeding colony and raised by their mothers on a 14 h light/10 h dark cycle. Tree shrew pups open their eyes about three weeks after birth. The day both eyes are open is the first day of visual experience (DVE). All procedures complied with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research and were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. Experimental groups were balanced to include both males and females, and avoided pups from the same parents wherever possible.

Five groups of animals ($n = 7$ per group) were used in this study (Fig. 1). Starting at 24 ± 1 DVE, a minus-lens wear group (LIM-2) wore a monocular −5 D (spherical power) lens for 2 days; the animals in this group also provided scleral mRNA for another study