Matrix Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Matrix Biology

journal homepage: www.elsevier.com/locate/matbio

Interclass small leucine-rich repeat proteoglycan interactions regulate collagen fibrillogenesis and corneal stromal assembly ☆

Shoujun Chen [a], Marian F. Young [b], Shukti Chakravarti [c], David E. Birk [a,*]

[a] Department of Molecular Pharmacology & Physiology, University of South Florida, Morsani College of Medicine, Tampa, FL, United States
[b] NIDCR, National Institutes of Health, Bethesda, MD, United States
[c] Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States

ARTICLE INFO

Available online xxxx

Keywords:
Corneal stroma
Biglycan
Lumican
Collagen
Fibrillogenesis
Stromal organization

ABSTRACT

The corneal stroma is enriched in small leucine-rich proteoglycans (SLRPs), including both class I (decorin and biglycan) and class II (lumican, keratocan and fibromodulin). Transparency is dependent on the assembly and maintenance of a hierarchical stromal organization and SLRPs are critical regulatory molecules. We hypothesize that cooperative interclass SLRP interactions are involved in the regulation of stromal matrix assembly. We test this hypothesis using a compound $Bgn^{-/0}/Lum^{-/-}$ mouse model and single $Lum^{-/-}$ or $Bgn^{-/0}$ mouse models and wild type controls. SLRP expression was investigated using immuno-localization and immuno-blots. Structural relationships were defined using ultrastructural and morphometric approaches while transparency was analyzed using in vivo confocal microscopy. The compound $Bgn^{-/0}/Lum^{-/-}$ corneas demonstrated gross opacity that was not seen in the $Bgn^{-/0}$ or wild type corneas and greater than that in the $Lum^{-/-}$ mice. The $Bgn^{-/0}/Lum^{-/-}$ corneas exhibited significantly increased opacity throughout the stroma compared to posterior opacity in the $Lum^{-/-}$ and no opacity in $Bgn^{-/0}$ or wild type corneas. In the $Bgn^{-/0}/Lum^{-/-}$ corneas there were abnormal lamellar and fibril structures consistent with the functional deficit in transparency. Lamellar structure was disrupted across the stroma with disorganized fibrils, and altered fibril packing. In addition, fibrils had larger and more heterogeneous diameters with an abnormal structure consistent with abnormal fibril growth. This was not observed in the $Bgn^{-/0}$ or wild type corneas and was restricted to the posterior stroma in $Lum^{-/-}$ mice. The data demonstrate synergistic interclass regulatory interactions between lumican and biglycan. These interactions are involved in regulating both lamellar structure as well as collagen fibrillogenesis and therefore, corneal transparency.

1. Introduction

The corneal stroma is enriched in small leucine-rich proteoglycans (SLRPs). There are 2 major classes of SLRPs in the corneal stroma. Decorin and biglycan, are class I SLRPs and lumican, keratocan and fibomodulin are class II SLRPs (Schaefer and Iozzo, 2008; Chen and Birk, 2011, 2013). These SLRPs demonstrate differences in their expression patterns in the developing and mature corneal stroma (Schaefer and Iozzo, 2008; Chen and Birk, 2011). Expression of both class I SRRPs is homogeneous across the corneal stroma. However, biglycan expression is low in the mature stroma while decorin expression remains high (Zhang et al., 2009). Class II SLRPs show both temporal and spatial differences. Lumican and keratocan are homogeneous across the corneal stroma at birth, but the expression of lumican is restricted to the posterior stroma after maturation (Chakravarti et al., 2006; Zhang et al., 2009; Chen et al., 2010). Fibromodulin expression in the corneal stroma is strongest at P14, but is decreased and restricted to the peripheral cornea with maturation (Chen et al., 2010). In addition, class I SLRPs have chondroitin sulfate/dermatan sulfate glycosaminoglycan (GAG) chains while class II SLRPs have keratan sulfate GAGs (Schaefer and Iozzo, 2008; Chen and Birk, 2013).

Human congenital corneal stromal dystrophy is the consequence of mutations in class I decorin that result in an altered protein core associated with abnormal stromal structure and function (Rodahl et al., 2006; Kim et al., 2011; Lee et al., 2012). Mutations in keratocan result in cornea plana with a change in corneal curvature resulting in altered refraction, but no significant fibril structural defects (Pellegata et al., 2000; Liu et al., 2003). Data has suggested a linkage between altered lumican expression and high myopia (Feng et al., 2013; Liao et al., 2013). Genetic mouse models support a role for SLRPs in these human conditions. In addition, the mouse models have demonstrated that SLRPs are critical regulators of collagen fibrillogenesis particularly the linear growth and lateral growth of protofibrils into mature fibrils (Chakravarti et al., 1998; Ezura et al., 2000; Chakravarti et al., 2003, 2006; Zhang et al., 2009; Chen et al., 2010; Chen and Birk, 2013). In a decorin-null corneal stroma there is a severe disruption of fibril structure and corneal function while the biglycan-null stroma has a phenotype comparable to wild type stromas (Zhang et al., 2009).

☆ This study was supported by NIH/NEI grant EY005129 and by the Intramural program of the NIDCR, NIH.

* Corresponding author at: Department of Molecular Pharmacology & Physiology, University of South Florida, College of Medicine, 12901 Bruce B. Downs Blvd., MDC08, Tampa, FL 33612-4799, United States. Tel.: +1 813 974 8598; fax: +1 813 974 5536.
E-mail address: dbirk@health.usf.edu (D.E. Birk).

0945-053X/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.matbio.2014.01.004

Corneal stromas deficient in class II lumican have a fibril phenotype restricted to the posterior stromal with both fibril structure and transparency disrupted in this region (Chakravarti et al., 2000, 2006). In contrast, deficiency in keratocan or fibromodulin is not associated with a fibril phenotype (Liu et al., 2003; Chen et al., 2010). These data suggested that there are dominant regulators and modulatory SLRPs in each class. We suggest that in the corneal stroma, decorin and lumican are dominant regulators while biglycan and keratocan/fibromodulin fine tune the regulatory activity both spatially and temporally.

Expression of SLRPs can be coordinated via gene clustering with class I decorin and class II lumican and keratocan cis-clustered on mouse chromosome 10 (Chakravarti and Magnuson, 1995; Danielson et al., 1999) and human chromosome 12 (Schaefer and Iozzo, 2008). SLRPs also have the potential to alter expression of the other class members, for instance, in the absence of decorin, biglycan expression can be increased (Zhang et al., 2009). In addition, the expression of lumican has been shown to drive the expression of keratocan (Carlson et al., 2005). Altered expression of both class I and class II SLRPs also was observed in a mutant decorin transgenic mouse model (Chen et al., 2011). Regulatory interactions across classes have been demonstrated by an increased severity of the tendon fibril structural phenotype in the absence of both biglycan and fibromodulin compared to either one alone (Ameye et al., 2002). Decorin and biglycan compete for a binding site on collagen I while fibromodulin and lumican compete for a different binding site (Pringle and Dodd, 1990; Svensson et al., 2000). This supports an interclass cooperativity in the regulation of fibril growth. Defining the network of regulatory SLRP interactions is essential to elucidate the roles in development, maintenance and regeneration of tissue-specific structures and functions.

In this work, we test the hypothesis that there are interclass regulatory interactions involving dominant and modulatory SLRPs. The roles of lumican and biglycan are investigated using lumican-null, biglycan- and compound lumican/biglycan-null mouse models and an analysis of corneal stromal matrix assembly. The data indicate a synergistic regulatory role for interclass interactions involving biglycan and lumican in regulation of corneal stromal matrix assembly. The interclass cooperation has significant impact on the development of cornea structure and transparency.

2. Results

2.1. Characterization of compound Lum/Bgn-null mice

To analyze interclass interactions involving class I biglycan and class II lumican in regulation of stromal matrix assembly, we generated a compound $Bgn^{-/0}/Lum^{-/-}$ mouse model by cross breeding of the single null mice. The mice were viable and demonstrated opacity of the cornea by gross examination (Fig. 1). The wild type and $Bgn^{-/0}$ corneas were clear while the $Lum^{-/-}$ cornea demonstrated gross opacity that was not as severe as that observed in the compound-null corneas.

SLRP expression was analyzed in the $Bgn^{-/0}/Lum^{-/-}$ corneas as well as in wild type, $Lum^{-/-}$, and $Bgn^{-/0}$ corneas using immuno-localization (Fig. 2) and immuno-blots (Fig. 3). The compound $Bgn^{-/0}/Lum^{-/-}$ P30 corneas were deficient in biglycan and lumican. The wild type corneas demonstrated homogeneous expression of biglycan, lumican and keratocan with an absence of fibromodulin. As expected, the $Bgn^{-/0}$ and $Lum^{-/-}$ corneas were null for lumican and biglycan respectively (Chakravarti et al., 1998; Xu et al., 1998). The $Bgn^{-/0}/Lum^{-/-}$ and $Lum^{-/-}$ corneas also demonstrated a decrease in keratocan. There was a significant up-regulation of fibromodulin expression in $Lum^{-/-}$ corneas (2.4 fold, $p = 0.04$), and a 1.7 fold increase in $Bgn^{-/0}/Lum^{-/-}$ corneas ($p = 0.059$). Decorin expression was not affected in any of the null mice. These mouse models will be utilized to address our

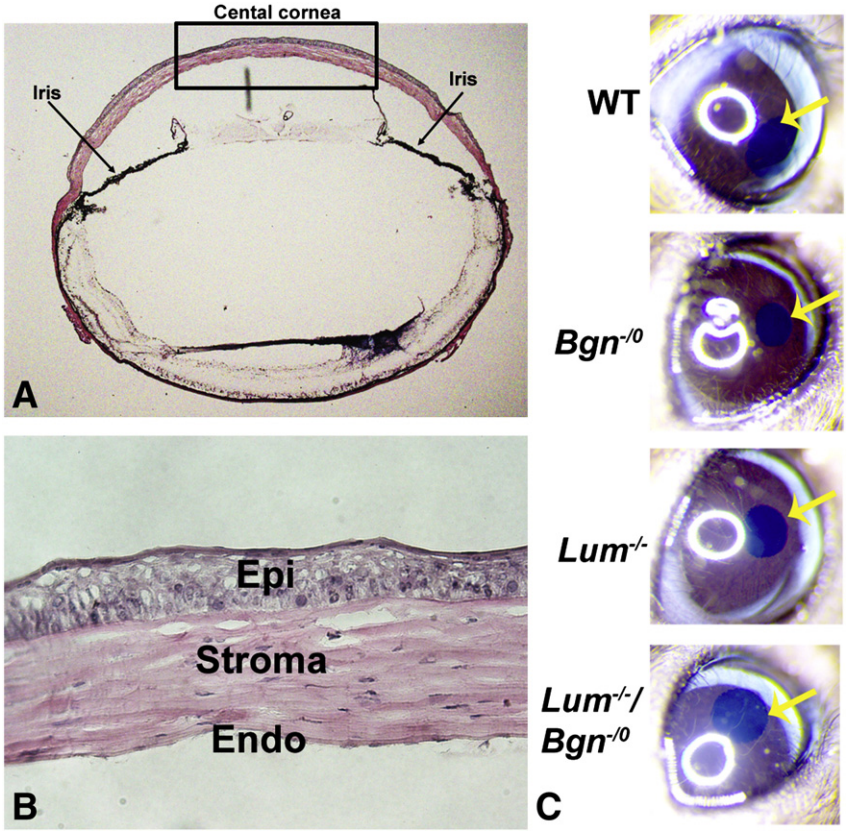

Fig. 1. Characterization of the compound $Bgn^{-/0}/Lum^{-/-}$ mouse cornea. (A) The black rectangle indicates the central cornea where all analyses were carried out. (B) The corneal stroma from the central cornea was analyzed. (C) Compound-null mice demonstrated corneal opacity with no opacity in $Bgn^{-/0}$ and wild type mice. $Lum^{-/-}$ mice showed less opacity than the compound mice. (The yellow arrow indicates the central cornea).

Please cite this article as: Chen, S., et al., Interclass small leucine-rich repeat proteoglycan interactions regulate collagen fibrillogenesis and corneal stromal assembly, Matrix Biol. (2014), http://dx.doi.org/10.1016/j.matbio.2014.01.004