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Excess biglycan causes eyelid malformation by perturbing muscle development and TGF-α signaling

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Abstract

Tissue morphogenesis during development is regulated by growth factors and cytokines, and is characterized by constant remodeling of extracellular matrix (ECM) in response to signaling molecules, for example, growth factors, cytokines, and so forth. Proteoglycans that bind growth factors are potential regulators of tissue morphogenesis during embryonic development. In this study, we showed that transgenic mice overexpressing biglycan under the keratocan promoter exhibited exposure keratitis and premature eye opening from noninfectious eyelid ulceration due to perturbation of eyelid muscle formation and the failure of meibomian gland formation. In addition, in vitro analysis revealed that biglycan binds to TGF-α, thus interrupting EGFR signaling pathways essential for mesenchymal cell migration induced by eyelid epithelium. The defects of TGF-α signaling by excess biglycan were further augmented by the interruption of the autocrine or paracrine loop of the EGFR signaling pathway of HB-EGF expression elicited by TGF-α. These results are consistent with the notion that under physiological conditions, biglycan secreted by mesenchymal cells serves as a regulatory molecule for the formation of a TGF-α gradient serving as a morphogen of eyelid morphogenesis.

Keywords: Biglycan; Eyelid morphogenesis; TGF-α; Muscle development; Keratocan promoter; Transgenic mouse

Introduction

In mammals, eyelid morphogenesis is characterized by the closure and fusion of the eyelid during development, which involves two major cellular events: the differentiation of ocular surface ectoderm to corneal, conjunctival, eyelid epidermal and glandular (lacrimal and meibomian) epithelia, and the migration and differentiation of periocular mesenchymal cells for the formation of eyelid stroma (Kao and Liu, 2003). Mouse eyelid formation begins at embryonic day 12 (E12), with folds of surface ectoderm adjacent to the developing eye. As it grows, the eyelid fold extends over the developing cornea, and periocular mesenchymal cells invade the eyelid stroma. The closure of the eyelid happens between E15.5 and E16.5, when the tips of the superior and inferior eyelid epidermis elongate toward the center of the eye and eventually cover the corneal surface (Findlater et al., 1993; Harris and Juriloff, 1986). Following epithelial fusion, the eyelid stroma grows by continued invasion and proliferation of mesenchymal cells, and a fusion line is formed between the superior and inferior eyelid epithelium

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to close the eye; thus, completing the formation of the eyelid protective barrier over the cornea. The mouse eyelids remain closed until 12–14 days postnatal. This process resembles the epithelial sheet migration of dorsal closure during *Drosophila* development (Glise and Noselli, 1997; Zhang et al., 2003), closure of choroid fissure of optic cup of vertebrates (Kurita et al., 2004; Macdonald and Wilson, 1996), and closure of fusion of chorioamniotic folds over platypus embryos (Hughes and Hall, 1998) and those of birds (Gilbert, 2003).

The eyelid serves as a protective barrier for the formation of the conjunctival sac and the development of the cornea and lens (Findlater et al., 1993; Harris and Juriloff, 1986). In normal mouse eye development, the commencement of eyelid formation coincides with the rapid growth of the eyeball beginning at E13.5. The formation of lid muscles, that is, orbicularis oculi, levator palpebral, and tarsal muscles, is essential for eyelid functions, which provide the adequate strength required to withstand the pressure exerted by the rapid growth of the eye. In addition, the tarsal plate at the end of the tarsal muscles provides the scaffold for the formation of meibomian glands that derive from epithelium at the tip of eyelids (Jester et al., 1989). The meibomian gland is a lipid-secreting gland that plays a pivotal role for the maintenance of tear film function. For example, meibomian gland dysfunction resulting from ectodermal dysplasia in human manifested severe exposure keratitis (Bron and Tiffany, 1998).

As in many other tissues, eyelid morphogenesis is also governed by bidirectional mesenchyme–epithelium interactions via growth factors, cytokines, and extracellular matrix (ECM) components during embryonic development. This is best illustrated by the eye open at birth phenotype (EOB) due to impaired epithelial cell migration in mouse strains in which the genes mediating signaling pathways of EGF, EGF receptors, and TGF-β superfamily members are ablated, for example, TGF-α, EGFR, cJun, MEKK 1, activin βB, and so forth (Li et al., 2003; Luetteke et al., 1993; Sibilia and Wagner, 1995; Vassalli et al., 1994; Zenz et al., 2003; Zhang et al., 2003). Thus, it is likely that genetic perturbations that impair epithelium migration can potentially cause eyelid malformation. However, it remains unknown whether mutation of these genes may impair the functions of periocular mesenchymal cells essential for eyelid morphogenesis.

Interestingly, failure in eyelid closure caused by spontaneous autosomal recessive mutations has been reported in several mouse strains, such as *eye-open-at-birth* (*eob*), *lidgap-Gates* (*lgGa*), *open-eyes* (*oe*), and *gaping lids* (*gp*) (Fujii et al., 1995; Juriloff et al., 1996; Stein et al., 1967; Teramoto et al., 1988). However, in each strain, the mutant gene responsible for EOB has not been identified (Juriloff et al., 1996, 2000; Kelton and Rauch, 1968). The obvious implication is that other molecular and cellular mechanism(s) besides epithelium migration may also be involved in eyelid morphogenesis. One of the many possibilities is a role for extracellular matrix components in eyelid morphogenesis, for example, proteoglycans, collagens, fibronectin, or laminin.

Proteoglycans belonging to the small leucine-rich repeat proteoglycan (SLRP) family, for example, decorin, biglycan, and fibromodulin, have been implicated as important molecules that bind and modulate the activities of growth factors and cytokines via their glycosaminoglycan moieties and core proteins (Kao and Liu, 2002; Iozzo, 1999; Reed and Iozzo, 2002). Biglycan contains two side chains of chondroitin sulfate/dermatan sulfate (CS/DS) glycosaminoglycans and is widely expressed by fibroblasts of interstitial connective tissues, mesothelial cells, and epithelial cells (Bianco et al., 1990; Dobra et al., 2000; Funderburgh et al., 1998). Like other members of the SLRP family, biglycan serves as a component of ECM and binds to a variety of other proteins, including growth factors such as TGF-β, TNF-α, and WISP-1 (Desnoyers et al., 2001; Hausser et al., 1994; Hildebrand et al., 1994); extracellular matrix proteins such as collagens I and V, fibronectin (Bidanset et al., 1992; Schmidt et al., 1987; Schonherr et al., 1995; Wiberg et al., 2003), and α-amyloid (Snow et al., 1995), apo E and apo B, α-dystroglycan, and phospholipase A2 type II (Bowe et al., 2000; Olin et al., 2001; Sartipy et al., 1998), and serum proteins such as heparin cofactor II (Fukushima et al., 1993; Hildebrand et al., 1994; Whinna et al., 1993). These various binding activities may account for the ability of biglycan to exert diverse functions in many tissues. For example, it has been suggested that biglycan may serve as a neurotrophic factor for the survival of neocortical neurons and participates in the regulation of neuronal growth, differentiation, and repair (Junghans et al., 1995; Koops et al., 1996). In addition, injection of biglycan into the brain apparently promoted the facilitation of learning (Huston et al., 2000). A recent study of rat neurons and C6 glioma cells revealed that a population of biglycan targets to the nucleus and may be involved in the regulation of neuronal cell division (Liang et al., 1997). Even though there is no conclusive evidence available, it is very likely that biglycan may also bind other growth factors, for example, TGF-α, activin βB, and so forth. Thus, biglycan is ideally situated to play a critical role in modulating the functions of growth factors in situ, and it is, therefore, conceivable that biglycan may play an important role in morphogenesis and tissue homeostasis.

We have created transgenic *Kera-Bgn* mouse lines using a 3.2-kb keratocan promoter (Liu et al., 2000), in which migrating periocular mesenchymal cells overexpress biglycan during embryonic development. The *Kera-Bgn* transgenic mice exhibited noninfectious eyelid ulceration causing premature eye opening and severe exposure keratitis. In this study, we present evidence illustrating that excess biglycan perturbs the formation of eyelid muscles and the migration of periocular mesenchymal cells by sequestering TGF-α