● PERSPECTIVE

Decorin treatment for reversing trabecular meshwork fibrosis in open-angle glaucoma

In this perspective, we discuss the use of an anti-fibrotic agent Decorin to treat established fibrosis associated with glaucoma originally published by Hill et al. (2015). Glaucoma describes a group of progressive optic neuropathies that have the potential to cause irreversible blindness, for which it is recognized that a main risk factor is raised intraocular pressure (IOP). Currently, there is no precise explanation of why high IOP causes retinal ganglion cell (RGC) death. Factors contributing to IOP-related RGC death include compromised retrograde axonal transport of target derived neurotrophic factors, leading to activation of apoptosis and mitochondrial dysfunction after the biomechanical and ischemic insult of optic nerve head compression. In open-angle glaucoma (OAG), increases in IOP occur when aqueous humour (AqH) outflow through the trabecular meshwork (TM) is reduced, usually as a result of progressive abnormalities in TM cellularity and extracellular matrix (ECM) levels culminating in TM fibrosis (Prendes et al., 2013). These cellular and ECM changes (which includes upregulation of collagen IV, laminin, and fibronectin) in the TM, together with altered TM cell contractility, result in TM dysfunction and ultimately loss of the tightly controlled AqH outflow. Currently, treatments for glaucoma are symptomatic and focus on the use of drugs formulated as eye drops to lower IOP either by reducing AqH production or increasing AqH outflow through non-TM pathways. Alternatively, surgical insertion of shunts through the TM can drain AqH directly to the exterior of the eye, although these often become blocked. For some patients it seems that primary open-angle glaucoma is a fibroproliferative condition and no current treatment addresses the TM fibrosis that is the predominant underlying causes of TM dysregulation.

The mechanisms that lead to TM dysfunction in OAG are multifactorial and may relate to dysregulated ocular inflammation since pathologically high levels of the pro-inflammatory cytokine transforming growth factor β (TGF-β) within the AqH and TM have been implicated. For example, some OAG patients have elevated levels of TGF-β1 and/or TGF-β2 in their AqH compared to age-matched patients with other forms of glaucoma (*e.g.*, primary angle closure glaucoma and uveitis-associated secondary glaucoma) and with cataracts (Prendes et al., 2013). Other studies have shown that it is the TGF-β2 isoform that is significantly elevated in eyes with primary OAG compared with non-glaucomatous eyes, and have shown elevated TGF-β1 and TGF-β3 isoforms in pseudoexfoliative glaucoma and in primary angle closure glaucoma (Prendes et al., 2013). TGF-β1 and TGF-β2 are known potent fibrogenic factors and, at pathologically high levels in the eye, can induce the overexpression of ECM proteins that lead to TM dysfunctions (Prendes et al., 2013). TGF-βs also prevent the breakdown of ECM by inhibiting matrix metalloproteinases (MMP) (Ahmed et al. 2014), creating a pro-fibrotic microenvironment within the TM. TGF-β is directly implicated in the pathogenesis of ocular hypertension since: (1) infusion of TGF-β2 for 14 days in an anterior eye segment perfusion culture model significantly increased IOP (Battacharya et al., 2009); (2) adenoviral gene delivery of human TGF-β2 into the anterior segment to the rodent eye reduced AqH outflow and increased IOP (Shepard et al., 2010); and (3) TGF-β1 gene delivery by adenovirus to the anterior segment of the rat eye increased IOP (Robertson et al., 2010). Thus, treatment of the TM in OAG with a TGF-β antagonist may help to ablate TM fibrogenesis, thereby perturbing the progression of OAG pathology.

The matrikine Decorin is a small leucine-rich proteoglycan that regulates cell proliferation, survival and differentiation by antagonizing a panel of growth factors and/or their receptors, including TGF-β, epidermal growth factor, vascular endothelial growth factor, hepatocyte growth factor and insulin-like growth factor-1. Decorin also 'decorates' collagen, interfering with collagen fibrillogenesis and stabilizing collagen fibres. In addition, Decorin enhances matrix metalloproteinases (MMP) activity by increasing levels of tissue plasminogen activator (tPA), thus enabling plasmin-dependent activation of MMP by cleavage of plasminogen to plasmin, and reducing levels of PAI-1 and tissue inhibitors of MMPs, further facilitating MMP activation. The fibrolytic actions of Decorin have been harnessed to attenuate the progression of tissue damage in other fibroproliferative pathologies, including juvenile communicating hydrocephalus (Botfield et al., 2013) and spinal cord injury (Ahmed et al., 2014), making Decorin an ideal candidate for treating TM fibrosis and progressive RGC loss in glaucoma.

Modelling raised IOP in OAG using intracameral (IC) injections of TGF-β: In the study by Hill et al. (2015), we used immunohistochemistry to visualize fibrosis and count RGCs, tonometry to measure IOP and visual evoked potentials (VEP) to measure function of the visual pathway, and found that repeated (twice a week) intracameral injections of TGF-β in adult rats promoted TM fibrosis, elevated IOP and led to RGC death and retinal dysfunction. Consistent with other fibrotic models of raised IOP, our rodent model generated a sustained and significant increase in IOP by 14 days compared with PBS controls. The baseline level of IOP (before any treatment) in our study was similar to that observed previously in rats (Robertson et al. 2010). However, overexpression of TGF-β by gene transfer (Robertson et al. 2010) led to greater levels of IOP (20 mmHg) compared to IOP levels (14 mmHg) achieved in our study, differences that maybe explained by the constant production of TGF-β through gene transcription compared with our discontinuous bolus regime of biweekly IC injections. One feature of our method was that TGF-β treatment for 17 days led to TM fibrosis and elevated IOP that persisted for at least 30 days despite withdrawal of TGF-β from this time point. To confirm that there was no natural resolution of the TM fibrosis and raised IOP (due to continued ECM turnover

in the TM) after the cessation of TGF-β treatment we used a control group in which intracameral injection of TGF-β injections for 17 days was followed by intracameral injection of PBS from 21–30 days with no resolution of IOP. Thus, in this *in vivo* model of TGF-β-induced TM fibrosis, IOP was increased by 17 days and sustained through to 30 days, resulting in 42% RGC death and associated functional VEP deficits, consequences that were similar to those seen in other experimental models of raised IOP (Belforte et al., 2010).

Of note, in the study of Hill et al. (2015) it was important to identify and account for any inflammatory reactions induced by the intracameral injections that may have perpetuated TM fibrosis. This was addressed by counting the number of $ED1^+$ macrophages situated within the proximity of the TM. The results showed that intracameral TGF-β and PBS did increase the number of macrophages in the angle compared with numbers in the intact control eyes. These results suggest that, since there were similar macrophage numbers in the PBS and TGF-β-treated groups, the induced differences between the two treatments were not a consequence of post-surgical inflammation.

Evaluating the utility of human recombinant Decorin hrDecorin for the treatment of OAG: Once the model was successfully established, hrDecorin was injected intracamerally after TM fibrosis was established to ascertain if the induced TM fibrosis and increased IOP could be resolved. hrDecorin significantly reduced the laminin and fibronectin levels within the fibrosed TM, an effect that was correlated with raised levels of MMP and their TIMP inhibitors. Thus, hrDecorin reversed the TM microenvironment back to MMP/TIMP ratios favourable for tissue remodelling, causing the dissolution of established ECM protein deposits in and around the TM, an anti-scarring response reminiscent of that seen to Decorin in models of spinal cord injury (Ahmed et al., 2013), hydrocephalus (Botfield et al., 2013) and conjunctival scarring for post-filtration surgery (Grisanti et al., 2005).

The study of Hill et al. (2015) in the OAG model also showed that the anti-scarring Decorin treatment lowered IOP when compared with controls *in vivo*, with associated enhancement of RGC survival. Primary retinal cultures containing RGC demonstrated that the neuroprotective effects of Decorin were indirectly related to the resolution of TM fibrosis and lowering of IOP rather than by direct actions on neurons. However, other studies have shown contradictory results on other cell types. For example, Seidler et al. (2006) reported an anti-apoptotic effect of Decorin on cultured fibroblasts by prevention of DNA fragmentation. By contrast, studies using cancer cells have convincingly demonstrated the ability of Decorin to induce cell death through apoptosis (Goldoni et al., 2008). Therefore, we suggest that the actions of Decorin are dependent on the cell type and their environment.

Conclusion: Our observations show that intracameral hrDecorin reverses established TM fibrosis and normalizes IOP, indirectly protecting RGC from progressive IOP-related death. Further confirmatory studies will be required to understand the exact mechanism of Decorin's fibrolytic actions within the TM but we believe that hrDecorin is an ideal candidate therapy to develop into a treatment for patients with OAG associated with TM fibrosis.

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