Experimental Eye Research xxx (2016) 1–10

Contents lists available at ScienceDirect

Experimental Eye Research

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

Research article

A single gene connects stiffness in glaucoma and the vascular system

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ARTICLE INFO

Article history:
Received 5 April 2016
Received in revised form
26 August 2016
Accepted in revised form 31 August 2016
Available online xxx

Keywords:
Calcification
Stiffness
Transgenic mice
Matrix Gla
Glaucoma

ABSTRACT

Arterial calcification results in arterial stiffness and higher systolic blood pressure. Arterial calcification is prevented by the high expression of the Matrix-Gla gene (MGP) in the vascular smooth muscle cells (VSMC) of the arteries' tunica media. Originally, MGP, a gene highly expressed in cartilage and VSMC, was found to be one of the top expressed genes in the trabecular meshwork. The creation of an *Mgp-lacZ* Knock-In mouse and the use of mouse genetics revealed that in the eye, *Mgp's* abundant expression is localized and restricted to glaucoma-associated tissues from the anterior and posterior segments. In particular, it is specifically expressed in the regions of the trabecular meshwork and of the peripapillary sclera that surrounds the optic nerve. Because stiffness in these tissues would significantly alter outflow facility and biomechanical scleral stress in the optic nerve head (ONH), we propose MGP as a strong candidate for the regulation of stiffness in glaucoma. MGP further illustrates the presence of a common function affecting key glaucomatous parameters in the front and back of the eye, and thus offers the possibility for a sole therapeutic target for the disease.

1. Introduction

1.1. Calcification and stiffness

Mineralization of the extracellular matrix (ECM) occurs when calcification deposits are formed into the extracellular space. Mineralization is initiated by the cell's release of membrane-bound matrix vesicles (MV) into the ECM, exposure to elevated Ca and Pi, and formation of calcium phosphate precipitates (Anderson, 2003; Kapustin et al., 2011, 2015). In the artery calcification, the majority of the calcification-competent extracellular vesicles are of exosomal origin. Once in the intercellular space, the released MVs associate with the ECM, interact with collagen and elastic fibrils, and by a still unknown mechanism serve as nucleation sites for the continuation of formation of hydroxyapatite nanocrystals (Kapustin et al., 2011, 2015; Krohn et al., 2016). Calcification is now known to be a highly regulated process. These MVs contain mineralization inducing proteins, such as alkaline phosphatase (ALP), MMP2 and annexins, as well as potent mineralization inhibitors, such Matrix Gla (MGP) and Fetuin-A (Krohn et al., 2016; Schurgers et al., 2013). Alteration in the production of these proteins by the cell would result in different levels of them being loaded into the vesicles and as a consequence, in different extents of calcification. Absence of *Mgp* in the arterial wall of mice results in premature death caused by massive arterial calcification (Luo et al., 1997). Matrix vesicles from vascular smooth muscle cells (VSMC) treated with calcification media show a dramatic reduction of MGP 48 h after treatment. (Kapustin et al., 2011).

Stiffness of a tissue, or resistance to deformation by a mechanical force, occurs when its cells and ECM undergo a number of biological changes which would lead to an overall compromise of its flexibility and rigidity. In the cell, stiffness is a physiological response that is required to resist an exogenous force. Stiffness is also associated with pathological conditions in a number of diseases such cardiovascular, cancer, chronic kidney disease (CKD), diabetes and glaucoma (Braunger et al., 2015; Kerr and Guerin, 2007; Mattace-Raso et al., 2006; Shim et al., 2015; Tiago et al., 2016; Visontai et al., 2005). Increased stiffness can be caused by a variety of cellular, cell-ECM and ECM hardening mechanisms. Per example, in endothelial cells lining the blood vessels, a stiffness response has been show to occur by the activation of the RhoA signaling pathway which leads to changes in cytoskeleton and focal adhesions to withstand the force (Collins et al., 2012). In these cells, an ECM signaling mechanism through integrins is also known to influence the response of mechanosensitive proteins and induce stiffness. In the vessels, stiffness induced by the sheer stress generated by the blood flow has been associated with elastin

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http://dx.doi.org/10.1016/j.exer.2016.08.022
0014-4835/

fragmentation, collagen deposition, matrix protein cross-linking, advance glycation end products, oxidative stress, angiotensin, dietary salt and a number of other factors (Zieman et al., 2005). Mineralization of the ECM leads inexorably to an increased stiffness.

Given the recent attention to the fact that stiffness in glaucoma-related tissues is associated with most, if not all glaucomatous conditions of elevated IOP (Braunger et al., 2015; Girard et al., 2009; Last et al., 2011; Nguyen et al., 2013) in this review we like to bring the reader's attention to the involvement of a single gene which would be mediating stiffness in glaucoma, as well as stiffness in the systemic arterial system. The gene is Matrix Gla (*MGP*), a potent mineralization inhibitor, known to be responsible for the physiological softness of cartilage, ossification of bone, and the mineralization and calcification/stiffness of arteries in the vascular system (Schurgers et al., 2013). In the eye, this gene has been found to be very abundant, and active only in the trabecular meshwork and peripapillary sclera (Borrás et al., 2015b), two tissues highly relevant to the development of glaucoma. *MGP* expression levels, as well as those of its activating enzyme λ-carboxylase, are reduced in the trabecular meshwork of primary open angle (POAG) patients when compared to normal, age-sex matched controls (Xue et al., 2007). Transcription of *MGP* is also reduced in primary human trabecular meshwork cells (HTM) treated with glaucomatous agents TGFβ1 (Vittitow and Borrás, 2004), TGFβ2, and dexamethasone (Xue et al., 2007). Mechanical forces of elevated IOP and stretch also alter expression of the *MGP* gene in human perfused anterior segment organ cultures and porcine trabecular meshwork (Comes and Borrás, 2009; Vittal et al., 2005; Vittitow and Borrás, 2004). In HTM cells, silencing the expression of *MGP* by siRNA, induces increase of the calcification marker ALP (Xue et al., 2007). When overexpressed in HTM cells, MGP reduced the calcification induction of BMP2 (Xue et al., 2006).

2. Association of vascular calcification and arterial stiffness

In the vascular system, calcification is associated with arterial stiffness. Early in 1997, before much of the calcification regulation was elucidated, experiments were conducted in rodents to demonstrate that accumulation of calcium in the media of the vessel wall would decrease aortic elasticity. The authors developed a rat model of calcium overload by treating rats with vitamin D and nicotine and produced 10–40-fold increase of calcium deposition in the elastic fibers of the media. After treatment, they measured stiffness of the arterial wall by elastic modulus, aortic pulse wave velocity (PWV) and isobaric elasticity. Their results showed that the stiffness of the arteries in the treated rats was significantly increased. Such increase was significantly correlated with the content of calcium in the wall (Niederhoffer et al., 1997). They therefore concluded that calcium overload on the medial elastic fibers is associated with decrease of aortic elasticity.

Association of calcification with stiffness is being nowadays observed in several major systemic diseases. Patients with CKD exhibit high mortality rate which does not seem to correlate with some of their risk factors such as elevated homocysteine, hypertension or diabetes, but rather with calcification of their blood vessels. Numerous reports indicate that, in CKD patients, medial calcification in the blood vessels occurs at an early age and with great severity. Measurement of stiffness in the calcified vessels of CKD patients as well as in those with end-stage renal disease by PWV demonstrated that not only the aortic stiffness was increased but that it was a strong predictor of cardiovascular mortality (Goodman et al., 2000; Kerr and Guerin, 2007; London et al., 2003; Robinson et al., 2005). Thus authors concluded that medial calcification and arterial stiffness play a major role in the development of CKD. In another disease, chronic obstructive pulmonary disease (COPD), patients were known to have increased aortic stiffness as measured by PWV. To investigate the relationship between aortic stiffness and calcification, a clinical study of 45 COPD patients absent of diabetes, renal or cardiovascular disease were subjected to thoraco-computed tomography for the determination of quantitative aortic calcium content. Their results showed a significant correlation of PWV values with aortic calcification. It was therefore concluded that aortic calcification was related to aortic stiffness in COPD patients (John et al., 2013).

3. Vascular dysfunction and arterial stiffness in glaucoma

The association of deficiencies in the vascular system with glaucoma, the vascular theory of glaucoma, has been around since the 19th century (Girkin, 2001). This theory proposes vascular health as an alternative risk factor to elevated IOP for glaucoma (Huck et al., 2014; Wirostko et al., 2009). Their thoughts are based on observations that some patients undergo glaucoma progression despite reduced IOP, while others do not develop glaucoma in the presence of elevated IOP (Heijl et al., 2002). Strong epidemiology data indicates that the higher incidence of glaucoma in African Americans correlates with their higher incidence of cardiovascular disease (Huck et al., 2014). Several other clinical studies directly measuring systemic and ocular fluid dynamics of blood flow have reconfirmed the association of vascular dysfunctions with risk factors for glaucoma (Choi and Kook, 2015). Together, the findings would indicate that other than IOP factors, such as genetic individual response to elevated IOP (Comes and Borrás, 2009) and/or proper functioning of the arterial vessels are bound to be involved in the development of glaucoma (Resch et al., 2009).

Arterial stiffness, or loss of arterial elasticity, is an important risk factor for cardiovascular diseases (Mattace-Raso et al., 2006). Arterial stiffness in glaucoma, has been investigated in a number of clinical studies using different measuring parameters. Using a non invasive high precision ultrasound wall-tracking system, it was shown that the distensibility coefficient sensitivity of the common carotid artery was significantly reduced in the glaucoma group versus sex/age-matched controls, while the stiffness was significantly increased (Visontai et al., 2005). More recently, using carotid-femoral PWV (CF-PWV) as the stiffness marker, the carotid artery of a group of pseudoexfoliation glaucoma (PEXG) patients showed significantly higher stiffness than that of the controls (Turkyilmaz et al., 2014). Based on this association between CF-PWV and PEXG, the authors proposed that the CF-PWV outcomes may be considered a risk factor for PEXG. Likewise, in a retrospective chart review, brachial-ankle PWV (baPWV) was analyzed in four groups: diabetes patients with POAG, POAG alone, normal tension glaucoma (NTG) and matched controls (Shim et al., 2015). Increased baPWV was positively associated with glaucoma in patients with diabetes, as well as in patients with only POAG and/or NTG. The authors proposed that arterial stiffness may contribute to the pathogenesis of glaucoma in diabetes patients.

4. Presence of stiffness in glaucomatous-relevant tissues

Elevated intraocular pressure (IOP) is the principal risk factor associated with the development of glaucoma (Kass et al., 2002). Physiological or elevated IOP is determined by the different resistances offered to the flow of aqueous humor by the trabecular outflow pathway (Rosenquist et al., 1989; Schuman et al., 1999). Dysfunction of this tissue by a variety of mechanisms would disrupt the facility of the aqueous humor flow and lead to pathological IOP and glaucoma. Investigators have determined the stiffness of the trabecular meshwork tissue from normal and glaucomatous

Please cite this article in press as: Borrás, T., A single gene connects stiffness in glaucoma and the vascular system, Experimental Eye Research (2016), http://dx.doi.org/10.1016/j.exer.2016.08.022