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Biomechanical aspects of axonal damage in glaucoma: a brief review[1]

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Abstract

The biomechanical environment within the optic nerve head (ONH) is complex and is likely directly involved in the loss of retinal ganglion cells (RGCs) in glaucoma. Unfortunately, our understanding of this process is poor. Here we describe factors that influence ONH biomechanics, including ONH connective tissue microarchitecture and anatomy; intraocular pressure (IOP); and

[1]This article summarizes the second part of a combined targeted session covering this topic at the March 2015 conference Astrocytes and Glaucomatous Neurodegeneration. For a summary of the first part see (Tamm et al., in preparation). This meeting was a follow-up to the 2010 meeting on the same topic, both of which were conducted as part of The Lasker/IRRF Initiative for Innovation in Vision Science. For more information about this conference, its participants and other review articles that originated from it see (Tamm and Dowling, 2016). A list of the other participants of the targeted session is provided at the end of this article

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cerebrospinal fluid pressure (CSFp). We note that connective tissue factors can vary significantly from one individual to the next, as well as regionally within an eye, and that the understanding of ONH biomechanics is hindered by anatomical differences between small-animal models of glaucoma (rats and mice) and humans. Other challenges of using animal models of glaucoma to study the role of biomechanics include the complexity of assessing the degree of glaucomatous progression; and inadequate tools for monitoring and consistently elevating IOP in animal models. We conclude with a consideration of important open research questions/challenges in this area, including: (i) Creating a systems biology description of the ONH; (ii) addressing the role of astrocyte connective tissue remodeling and reactivity in glaucoma; (iii) providing a better characterization of ONH astrocytes and non-astrocytic constituent cells; (iv) better understanding the role of ONH astrocyte phagocytosis, proliferation and death; (v) collecting gene expression and phenotype data on a larger, more coordinated scale; and (vi) developing an implantable IOP sensor.

1. Introduction

Previous controversies as to whether and how intraocular pressure (IOP) is important in the pathogenesis of glaucoma have faded over the past 20 years. There is now broad consensus among both scientists and clinicians that IOP and the mechanisms by which the relevant tissues respond to IOP are critical in the pathogenesis of glaucoma. It is also recognized that glaucomatous damage can be initiated at any level of IOP; further, additional genetic and/or environmental risk factors contribute to the eye-specific risk of developing the disease, some of which are IOP-related and some of which are IOP-independent. Nonetheless, while the biomechanical processes by which IOP contributes to retinal ganglion cell (RGC) axon damage within the optic nerve head (ONH) are considered to be a fundamental part of the pathogenesis of glaucoma, they are not fully understood.

This article focuses on the role of ONH biomechanics in glaucoma. For the purposes of this discussion we define the ONH to include the tissues within and immediately surrounding the scleral canal. In this article we identify fundamental questions and discuss the usefulness (including benefits and limitations) of various animal models to answer these questions. We will also identify important developments over the last five years and propose experiments for the next five years. Because the Lasker Meeting sessions dealing with astrocytes (Tamm et al., in preparation) and ONH biomechanics (the present report) were combined into a single discussion session, some overlap between the articles devoted to each session is necessary to ensure that each summary is complete.

2. Questions related to ONH structure and glaucoma susceptibility

We first focus on connective tissues elements of the ONH, principally the lamina cribrosa (LC) and peripapillary scleral (pp-sclera), since these elements provide the majority of the structural support to the ONH in the face of IOP. Are all humans formed equally with respect to the quality and quantity of their ONH connective tissues? The answer is almost certainly “no” for any genetically diverse species, including humans. Moreover, the

likelihood of intra-individual (between-eye) and regional (within-eye) differences in connective tissue properties and glaucomatous damage susceptibility must be borne in mind.

ONH connective tissue variability arises from both macro- and micro-architectural factors. Macro-architectural (anatomical) factors include the size and shape of the scleral canal; the thickness of the LC and pp-sclera; and LC beam and pore dimensions. Microarchitectural factors include the density, quantity, orientation and molecular nature of relevant fibrillar and non-fibrillar extracellular matrix (ECM) components, which ultimately depend on the ability of fibroblasts and astrocytes to robustly maintain and remodel ECM components. The micro-architectural factors determine the local tissue material properties. We must also recall that each of these factors likely change with age and disease state. Taken together, differences in these factors will influence the biological, mechanical and physical properties of the ONH connective tissue elements.

The combination of macro-architecture (anatomic) features and material properties defines the overall structural stiffnesses of ONH connective tissues, which in turn dictate the magnitude of global and local deformations (strains) experienced by the ONH tissues for a given pressure-related load, including loads due to IOP, cerebrospinal fluid pressure (CSFp) and possibly even orbital pressure.

Apart from determining the material properties of a given connective tissue, microarchitectural features also influence diffusion coefficients (including those of signaling molecules) and the ease with which monocytes and macrophages pass into and within those tissues. These issues should be of special importance within the LC beams, as the lamina is the only location in the central nervous system where astrocyte processes do not directly contact local capillaries (Burgoyne, 2011; Hogan et al., 1971). Because the RGC axons have no direct blood supply within the LC, it is believed they are dependent upon local astrocytes for nutrient delivery. The ECM of LC beams, as well as the basal laminae of the LC beam endothelial cells and astrocytes, are thus potential barriers to nutrient delivery to the RGC axons within this region.

To further complicate the situation, signaling molecules that cause cells to modify ECM quality or quantity, such as those of the transforming growth factor-β (TGF-β) family, can bind to ECM components and be activated and released from the ECM in response to mechanical cues. Consequently those molecules likely play an important role in ONH biomechanics and its changes with aging and glaucoma (Burgoyne and Downs, 2008; Tamm et al., in preparation).

ONH connective tissues stiffen with age (Fazio et al., 2014a; Fazio et al., 2014b; Grytz et al., 2014), although it is not clear if the individual components of those tissues stiffen at different rates. The process may not progress at comparable rates in all humans, as there is strong evidence that it is influenced by genetic factors (Fazio et al., 2014b). It is currently assumed that the LC contributes to RGC axon susceptibility within the ONH at all levels of IOP and at all ages, but the critical molecular components of this susceptibility remain unknown. Some humans develop glaucomatous optic neuropathy at low (“normal”) levels of IOP, while others can be followed with IOPs in the 30 – 40 mmHg range without evidence

of the neuropathy for 5 to 10 years (there currently are no studies that extend beyond 10 years of follow up). Are there specific biological/mechanical/physical properties of the ONH connective tissues that can wholly or partially explain these differences in susceptibility?

With these concepts in mind, another question is: What are “good” and “bad” properties of the ONH connective tissues? One would presume that “good” properties are those that prevent reactive changes in ONH astrocytes and microglia, keep monocytes and macrophages out of the ONH, and facilitate normal function of RGC axons. Still, what is the exact role of those cells in glaucoma? Do glial cells become reactive to protect from neuronal damage or do they accelerate it? Is it better to have rigid or compliant ONH scleral connective tissue elements to achieve all or some of those goals? Alternatively, is a mismatch of a stiff LC and compliant sclera best? What makes for a robust versus a weak LC in terms of axonal preservation? These important questions remain unanswered.

A related point concerns the role of focal laminar defects (“pits”) (Irvine et al., 1986; Ohno-Matsui et al., 2013). While it might be argued that a “good” LC is one without pits, pits might instead be beneficial strain relievers for the rest of the ONH connective tissue elements. Clinically, some patients have focal LC defects, and although RGC axons in the pit area will sustain damage regardless of the IOP, the rest of the ONH and its optic nerve axons remain stable. Overall, better phenotyping of the biomechanical responses of the ONH connective tissues along with improved molecular characterization is required to allow their contributions to RGC axon susceptibility to be determined.

Years ago, two laboratories independently demonstrated that in both monkeys (non-human primates) and humans the connective tissue beams of the LC are made of collagen I, III, and IV (the latter especially in the endothelial and astrocyte basal lamina), and elastin (Hernandez et al., 1990; Morrison et al., 1990; Morrison et al., 1988). The respective roles of those molecules for the biomechanical properties of the LC are as yet unclear. If monkeys could be genetically modified, the effects of changing collagen and elastin in the ONH scleral connective tissue elements (including the LC) could be assessed. Unfortunately, as of now, such genetic modifications are only feasible in mice in which the ONH contains a cellular (glial) rather than a connective tissue LC (Sun et al., 2009).

It should be noted that the biomechanical environment of the LC is intricately linked to that of the pp-sclera (Burgoyne et al., 2005; Sigal et al., 2009a, b), since the pp-sclera establishes the boundary conditions for the LC beam insertions into and through the scleral canal wall. The magnitude of pp-scleral load delivered to the LC is likely larger than the load due to the translaminar pressure difference (defined as IOP minus retrolaminar tissue pressure, which is close to or slightly above CSFp) at all levels of IOP. Currently, experiments designed to address the role of scleral stiffness in RGC axon insult in glaucoma are most common in rodent experimental glaucoma models. For example, experimental cross-linking of the scleral ECM increased stiffness and susceptibility to RGC damage in mice (Kimball et al., 2014). Preliminary data published as abstract indicate a potentially different outcome in rats (Gonzalez P, et al. IOVS 2015; 56: ARVO E-Abstract 2006). Here, virally-mediated overexpression of bone morphogenetic protein 2 (BMP2), presumably acting by increasing the rigidity of the sclera, partially protected against IOP-induced optic nerve damage. Other

approaches may stiffen or loosen the sclera by alternative methods. Moreover, genetically modified mouse models are available which are deficient in specific ECM molecules or signaling pathways (including TGF-β). Taken together, rodent animal models are being used to address the biological and/or mechanical influences of the pp-sclera on glaucomatous ONH damage. However, because of their lack of a connective tissue LC (Tamm et al., in preparation) rodent models must be complemented with experiments in animals with a connective tissue LC.

3. Animal models of glaucoma

Since the Lasker meeting, a series of review articles on animal models in glaucoma have been published which are a recommended reference for the section that follows (Ethier et al., 2015). Even though there is currently no animal model that mimics human glaucoma and its tremendous variability, models, when correctly implemented and interpreted, are valuable for addressing specific questions in glaucoma. There are, however, general limitations that need to be addressed

1. There is very little consensus on how to "stage" glaucoma in any species, other than to quantify retinal ganglion cell (RGC) somata or orbital optic nerve axons. The same endpoint defined in this way can be generated quickly at high IOP or slowly at low IOP. It is possible that different damage mechanisms are at work in each setting, some of which are not relevant for glaucoma in humans.

2. Even at a given level of RGC somata or optic nerve axon loss in an eye, there may be profound regional differences, i.e., some ONH regions within an optic nerve may appear normal, while other regions show early insult, and still other regions show profound alteration. Careful phenotyping is required to identify these differences, which may include molecular, cellular, and tissue-specific features. It is hoped that the effort to phenotype and stage the neuropathy (both its neural and connective tissue components) will ultimately lead to mechanistic insights, and *vice versa*.

3. There is great difficulty characterizing the magnitude of IOP insult in a given eye, regardless of species. For example, recent work indicates that IOP is highly dynamic, a feature not captured by standard "snapshot" IOP measurements (Downs, 2015). Continuous telemetric IOP monitoring may reveal which aspects of a given eye's IOP profile are most important in models of glaucoma. Telemetric systems have been developed for monkeys and may be available for mouse and rat eyes in the near future.

4. To control IOP insult in animal models is difficult, which hampers the ability to perform studies designed to assess the effect of non-IOP-related risk factors, since such studies would ideally incorporate identical IOP insults in all treatment groups. Eventually the control of IOP insult should include programmable short- and long-term IOP fluctuation. This issue has led several groups to concentrate their efforts on manometer-controlled, short-term IOP elevations as a means of studying various factors contributing to ONH susceptibility (Crowston et al.,

2015). There are also efforts to create longer-duration IOP-control systems in rodents (Bello et al., 2016).

The advantages and limitations of specific animal models are summarized below. For more detailed information the reader is referred to the articles of a special journal issue recently published on this topic, mentioned above (Ethier et al., 2015).

Monkey

Similar to the human, monkeys are mammals of the order Primates. Their ONH is anatomically and functionally very much like the human, including having a connective tissue LC, which is important when considering biomechanical factors in the ONH and comparing them with the situation in human. The size of monkey species used in biomedical research (frequently from the genus macaques) allows certain experiments to be done that are more difficult to perform in smaller animal models. Because of the fact that it is essentially (i.e. practically) impossible to obtain human tissues at the transition from ocular hypertension to the earliest stage of alterations/damage in glaucoma, the monkey model is, and will remain, very advantageous for longitudinally studying early ONH damage induced by high IOP.

Significant limitations include the cost, accessibility, and the difficulty of housing and working with monkeys. Monkeys share with humans the wide range of genetic variability that is especially problematic for studies involving genome-wide assessment profiles of proteomes or transcriptomes. Genetic engineering as performed in mice or rats is not feasible, though the use of viral vectors for gene transfer is increasing. While IOP telemetry has been achieved, the ability to control the magnitude and character of IOP insult, as noted above, has not.

Rats and mice

These rodent species have been widely used because they are available in inbred strains with genetically identical individuals, an advantage that theoretically minimizes the number of animals needed to obtain significant data after experiments. In addition, the fact that they can readily be genetically manipulated has revolutionized the field of biomedical research over the past decades. The Knockout Mouse Project (KOMP), a trans-NIH initiative, aims at generating a comprehensive and public resource comprised of mouse embryonic stem (ES) cells containing a null mutation in every gene in the mouse genome. Rats and mice are cheap, and easy to breed and house. Possibly the largest limitation to the use of rats and mice is their ONH anatomic differences compared to humans. Specifically, in the rat and mouse eye, the scleral canal is surrounded by pp-sclera, but contains no connective tissue beams forming a LC (Dai et al., 2012; Howell et al., 2007; Johansson, 1987; May and Lütjen-Drecoll, 2002; Sun et al., 2009). Just posterior to the pp-sclera, astrocytes form an enmeshing network termed the “glial lamina” through which the RGC axons pass (Dai et al., 2012; Howell et al., 2007; Sun et al., 2009). Despite those differences in ONH structure, increased IOP induces ONH axonal degeneration in rats and mice indicating that the presence of a LC is not essential for the development of glaucomatous damage, at least in these species. In addition, there are differences to primates with regards to the ONH vasculature. In mouse ONH the central retinal vessels obliquely enter the optic nerve at the

level of the sclera (May and Lütjen-Drecoll, 2002). In contrast, in human and monkey they enter the optic nerve posterior to the LC (May, 2008). The rat ONH contains two scleral openings: a superior neurovascular opening and an inferior arterial opening, separated by a thin connective tissue ("scleral sling") from the superior opening. The superior opening contains a vascular plexus that passes through the sclera and partially surrounds the optic nerve. The inferior scleral opening contains the central retinal artery and three long posterior ciliary arteries (Pazos et al., 2015a, b).

Rabbits

This species is more accessible and easier to breed and house than monkeys, and has considerable larger eyes than rats or mice, a fact that facilitates experimental procedures. Rabbits with hereditary congenital glaucoma and optic nerve damage in response to high IOP have been characterized (Bunt-Milam et al., 1987a, b). At the ONH, a poorly developed LC has been described with some connective tissue beams that radiate from the central retinal vessels to the optic nerve sheath (Flage, 1977). A significant difference to the human is the fact that rabbit RGC axon myelination begins on the retinal surface before the axons exit the eye (Vaney, 1980). In humans, myelination of optic nerve axons on the retina is rarely observed and appears to protect against IOP insult (Toh et al., 2011). A similar protective effect of myelination in the rabbit would be a confounding factor when using this species as animal model to assess ONH damage in glaucoma. Other limitations include the presence of a tremendous inflammatory response (Bito, 1984), and large anatomic differences in the vascular supply compared to primates. In the rabbit, retinal vessels are confined to a broad horizontal band coincident with the area covered by myelinated axons (De Schaepdrijver et al., 1989; Rohen, 1954).

New Animal Models

Pig, cat and dog eyes have a well-developed LC (Coudrillier et al., 2016a; Coudrillier et al., 2016b; Fatehee et al., 2011; Grozdanic et al., 2010; Radius and Bade, 1982a, b), but their ONH vascular anatomy is considerably different to that of the human (De Schaepdrijver et al., 1989; May, 2008). Glaucoma commonly caused by anterior segment dysgenesis and high IOP is a frequent condition seen in cats and dogs (McLellan and Miller, 2011; Pizzirani, 2015). Colonies of cats and dogs with hereditary glaucoma are available (Grozdanic et al., 2010; Kuchtey et al., 2011; Narfstrom et al., 2013). The ONH of the guinea pig has laminar beams that are radially oriented, emanating from a central fibrovascular stalk (Morrison et al., 1995; Ostrin and Wildsoet, 2016). The guinea pig retina is avascular (De Schaepdrijver et al., 1989), which is a substantial difference to the human. Spontaneous glaucoma has not been reported for guinea pigs (Williams and Sullivan, 2010) and it is still uncertain whether or not animals of this species would develop glaucomatous damage after experimental elevation of IOP. A promising novel animal model for glaucoma research may be the tree shrew (family Tupaiidae), a non-rodent, primate-like species (Cao et al., 2003) that has been widely used in myopia research. The animals are easy to breed, inexpensive and their use in myopia research provides substantial knowledge of their scleral biology as well as the ability to study, in the same species, myopia, glaucoma and myopic eyes in which chronic IOP elevation has been introduced. A first characterization of the LC and scleral canal, and its ECM components in the normal tree shrew eye is available (Albon et al., 2007). In more

recent preliminary studies, magnetic beads were used for chamber angle occlusion in tree shrew eyes to achieve unilateral IOP elevations into the mid-20 mmHg range (Zhan W., et al. IOVS 2015; 56: ARVO E-Abstract 2428). After several weeks, thickness of the retinal nerve fiber layer was reduced in the treated eye. Moreover, *in vivo* SD-OCT imaging showed ONH and LD cupping in the glaucomatous eye of all animals, which was confirmed by 3-D histomorphometry. A high-quality genome sequence and the annotation of Chinese tree shrew are available (Fan et al., 2013). Still, questions remain how easily they can be genetically engineered.

In general, the differences between animal models, although a source of complexity, have the potential to inform us about the pathogenesis of glaucoma. There is no single model that best replicates human glaucoma and a diversity of models is needed to address the diversity of research questions in glaucoma.

4. Fundamental questions to be answered in animal models

Using the models discussed above, there are a number of questions that should be addressed to increase our understanding of glaucoma.

(1) Are ONH astrocytes fundamentally different from astrocytes in other regions of the central nervous system (CNS)? Glial lamina astrocytes of the mouse optic nerve appear to differ in structure from white matter astrocytes in other parts of the CNS, since the processes of individual astrocytes are far-reaching to span most of the width of the optic nerve (Sun et al., 2009), a structural peculiarity that could be explained by the unique spatial and biomechanical requirements of ONH astrocytes. It is unclear, but not necessarily unlikely, that ONH astrocytes also differ in their gene expression profile from other populations of astrocytes in the CNS. In order to address this important question and to analyze their gene expression profile, ONH and other astrocytes from different species, including humans, could be isolated, dissociated and sorted using cell-specific markers. This approach should be feasible for the mouse glial lamina, since mice are available with astrocytes that specifically express fluorescent markers such as GFP that could be used for cell sorting. A comparable approach could be used to isolate and molecularly characterize subpopulations of ONH astrocytes (if there are any). To achieve this goal, isolated cells could undergo transcription profiling using single-cell RNA sequencing (scRNA-seq).

Do ONH astrocytes of the normal and glaucomatous glial lamina (in species that lack a connective tissue LC) differ in their gene expression pattern from those that cover the LC beams in species that have a connective tissue LC? To date, while gene array studies have been performed in the ONH of animals with experimental glaucoma, the studies have only investigated the total RNA expression profile which included all cell types (Howell et al., 2011; Johnson et al., 2011; Johnson et al., 2007).

(2) Why do some animal species have a connective tissue LC with beams containing capillaries covered by astrocytes while others have a glial lamina consisting of astrocytes and capillaries only, without additional connective tissue structural support? One hypothesis for the lack of a collagenous LC in the mouse is that in a relatively small, normal, healthy

eye at normal IOP, the additional structural support from collagenous beams may be unnecessary. Supporting evidence for this assumption comes from studies showing that single astrocytes are able to span the entire scleral canal in mice (Lye-Barthel et al., 2013; Sun et al., 2009). It would be important to know if this is also true in the rat, where the scleral canal is bigger. Some observations appear to support this concept but are not definitive on this point (Dai et al., 2012).

RGC axonal transport is obstructed within the ONH in response to high IOP, irrespective if there is a LC or a glial lamina. Thus, a LC obviously does not protect the RGC axons from transport difficulties. Could the evolutionary role of the LC be related to the size of the canal or the fact that the LC connective tissue beams are formed around the ONH capillaries? Connective tissue could prevent mechanical obstruction of capillaries. Alternatively, connective tissue might constitute a barrier against macrophage evasion from capillaries or change the activity of growth factors; for example, TGF-β being inactive when bound to ECM. The genes that control the development of a collagenous LC have not been identified and there are no human diseases that result in the complete absence of a LC. There are, however, congenital and acquired laminar pits (Irvine et al., 1986; Ohno-Matsui et al., 2013), which appear to be focal absences of the laminar insertion and can span several clinical clock hours.

(3) Are astrocytes and LC beam fibroblasts the only cells that synthetize the ECM of the LC beams? Astrocytes are characterized in part by their basal lamina and transmission electron microscopy studies have demonstrated the basal lamina to be integral with the LC beam ECM. During embryonic development of the human eye, the beams of the LC are formed in the second and third months of gestation by astrocytes that orient themselves perpendicularly to the ganglion cell axons (Morrison et al., 1989). The basal laminae of the astrocytes form the outer boundaries of the beams and label for laminin and collagen type IV. At the same time, neural crest-derived mesenchymal cells build the sclera while subsequent migration of these mesenchymal cells into the LC beams is presumably prevented by the astrocyte basal laminae. Still, around the nerve head (within the developing peripapillary sclera) there are mesenchymal cells that are very efficient in generating extracellular matrix, most likely more efficiently than the astrocytes (Morrison et al., 1989), Years ago it was proposed that in addition to astrocytes characterized by a basal lamina, there is another type of ONH cells, the so-called lamina cribrosa cells (lamina cribrocytes), which lack a typical basal lamina as seen by transmission electron microscopy of the LC (Hernandez, 2000) and in cultures from explants (Hernandez, Igoe and Neufeld, 1988). Lamina cribrocytes might indeed constitute a population of neural crest-derived mesenchymal cells that constitute the laminar beam fibroblasts in a mature eye. A comprehensive molecular characterization of LC cells should be performed in embryonic human eyes to differentiate localization and time of migration into the LC of neural crest-derived cells (scleral fibroblasts and/or possible lamina cribrocytes) versus neural tube-derived astrocytes.

(4) In the human eye, the LC ECM is rudimentary in premature eyes and its development is not completed until the late teenage years (Morrison et al., 1989). There is the distinct possibility that through human development the astrocytes experience physiologic levels of

biomechanical stress and strain and, in response, lay down collagen as a means of coping with it. It is of yet unclear if laminar beam capillaries differentiate first and then astrocytes migrate to them and lay down ECM including a basal lamina, or if the LC ECM forms at the same time as the capillaries. Careful studies to address those questions are warranted.

5. Recent developments

Over the last five years, an extensive body of research has been conducted in both animal models and humans leading to the following (selected) key developments:

1. In the area of biomechanics, important characterization of very early morphometric changes in the monkey ONH unilateral experimental glaucoma model has occurred (Yang et al., 2015). Briefly, the monkey ONH surface, LC, scleral canal and pp-sclera are compliant structures, demonstrating modest deformations that are reversible through an IOP range of 0 to 45mmHg. In a given eye, confocal laser scanning tomographic (CSLT)- and spectral domain OCT (SDOCT)-detected ONH surface compliance is not representative of underlying LC compliance. In subsets of eyes, scleral canal expansion and outward bowing of the pp-sclera are part of the ONH response to IOP. ONH finite element models in bilateral normal animals suggest that contralateral eyes exhibit similar mechanical behavior, and that local mechanical stress and strain correlate highly with local laminar connective tissue volume fraction (a measure of connective tissue density). Initial descriptions of decreased LC beam thickness and increased pore diameters within the superior and inferior scleral canal of monkey and human eyes were recently confirmed in 21 normal and control monkey eyes using three dimensional histomorphometric reconstruction (3D HMRN) techniques that regionalize the ONH tissues relative to the axis between the fovea and the center of Bruch's membrane opening (the FoBMO axis).
2. In the area of glial cells, the discovery that resident astrocytes have a phagocytic role in the normal ONH is very important (Davis et al., 2014; Nguyen et al., 2011). Changes in phagocytosis in reactive astrocytes might contribute to ONH axonal damage. Another important finding is the causative role of optic nerve monocytes/microglia for optic nerve degeneration in the mouse model of hereditary glaucoma (Howell et al., 2012).

6. Proposed experiments for the next five years

The following experiments are proposed to provide more knowledge on the role of biomechanics for astrocyte biology.

1. Create a systems biology description of the ONH including, but not limited to, RGC axons, constituent cells (astrocytes, endothelia, pericytes, fibroblasts, oligodendrocytes) connective tissue morphometry, biomechanics and blood flow.
2. Address the question of how astrocyte connective tissue remodeling and reactivity in response to IOP influences the ability to provide trophic support to the RGC axons within the ONH. The retina appears to be very different from the

ONH with regard to astrocyte reactivity. Extreme retinal astrocyte remodeling on the retinal surface occurs after retinal injury, but without any obvious signs of RGC death (Luna et al., 2016).

3. A complete molecular and morphometric characterization of the ONH astrocytes and non-astrocytic constituent cells is needed. This would include, but not be limited to, creating a catalog of molecular markers (through characterization of both RNA and protein changes) that astrocytes exhibit differentially in different contexts in response to tissue change. Moreover, it needs to be investigated whether susceptibility to astrocyte reactivity and RGC loss changes with age. Are there differences in absolute and relative aging? Some ECM proteins are made early and have hardly any turnover. Do these proteins contribute to an age-dependent susceptibility to damage? Age-related differences in ONH susceptibility to elevated IOP are under study in mice, rat and monkey experimental glaucoma models.

4. The role of ONH astrocyte phagocytosis (Davis et al., 2014; Nguyen et al., 2011) needs to be explored in more detail. Astrocyte reactivity may reduce the cells' ability to perform this essential function, leaving the RGC axons more susceptible to glaucomatous insult.

5. Proliferation and death of ONH astrocytes in the normal eye and in response to injury needs to be investigated. Is proliferation a consequence of IOP-induced mechanical strain and/or axonal degeneration? If astrocytes divide early in response to acute or chronic IOP elevation, would blocking of proliferation be beneficial or detrimental? If astrocytes die, can they regenerate? Do astrocytes migrate away from the LC beams (and their connections to capillary nutrient delivery) as part of "glaucomatous" reactivity?

6. Gene expression and phenotype data need to be collected on a larger, more coordinated scale. Databases of the genetic changes in all models need to be established.

7. There is need for better alignment of staging and outcome measures in all animal models of glaucoma.

8. The development of an implantable IOP sensor that is able to gather data at sufficiently high frequency, and is sensitive enough to determine IOP changes in individual patients/animals, is highly desirable. The data would allow a better understanding of how IOP affects disease progression. One of the issues with trying to classify the disease into various susceptibility phenotypes is that, because we don't know much about one of the main drivers of the disease (IOP), we cannot effectively control for IOP. This in turn makes it difficult to isolate and understand other risk factors. Such an approach would ideally incorporate both IOP characterization and IOP control. Experiments could be designed to assess the effects of pressure (sustained, transient, spikes, prolonged, etc.) on retinal neurons and glial cells. In other experiments, the magnitude and character of IOP insult could be controlled and other risk factors could then be varied.

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