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Biomarkers of primary open-angle glaucoma

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Abstract

Primary open-angle glaucoma (POAG) is a primary neuronal disease of the optic nerve without a definable cause, and is often associated with increased intraocular pressure. Worldwide, POAG is the second leading cause of blindness; there are 45 million people today with POAG and bilateral blindness is present in 4.5 million of these. In order to elucidate the possible etiologic factors in POAG, we have cataloged all known biomarkers in the aqueous humor, trabecular meshwork, optic nerve and blood into four categories, namely extracellular matrix (ECM), cell signaling molecules, aging/stress and immunity-related changes. We present a theoretical model to show possible signaling pathways of the ECM, cell signaling and innate immune response through activation of Toll-like receptor 4. Our article suggests that ECM and innate immune biomarkers are the lead candidates for developing the 'POAG biomarker signature'. We suggest that current research is critical to pinpoint the causes of the disease so that new treatment modalities can become available for better regulation of the intraocular pressure and neuroprotection of the optic nerve.

Keywords

biomarker; intraocular pressure; neuroprotection; primary open-angle glaucoma

Primary open-angle glaucoma (POAG) is a chronic, degenerative optic neuropathy characterized by concurrent loss of retinal ganglion cells and their axons, resulting in optic nerve cupping and visual field loss (Figure 1) [1,2]. In POAG, the iridocorneal angle is open (unobstructed), but the aqueous outflow through the trabecular meshwork (TM) is usually diminished as a result of aging and/or POAG causative factors [3]. POAG is the most common type of glaucoma, the second leading cause of blindness worldwide and the leading

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cause of blindness in African-Americans [1]. Most POAG patients have increased intraocular pressure (IOP); however, some patients have IOP in the normal range and are classified as ‘normal-tension glaucoma’. In our opinion, POAG encompasses both patients with normal tension, as well as patients with an increased IOP. Results of a 2010 ocular hypertension treatment study provided strong evidence that IOP is associated with the progression of ocular hypertension to POAG, as well as evidence that other factors including greater cup-to-disc ratio, lesser central corneal thickness and age are also risk factors [4]. If all these factors are present, an individual’s risk for POAG is greater than 33% in 5 years [5]. For every 1-mmHg increase in IOP, there is a 10% increase in relative risk of POAG [6].

The major ocular risk factors for POAG are IOP elevation and aging. The prevalence of POAG increases from 0.02% at ages 40–49 years to 2–3% for persons over the age of 70 years, while the incidence of ocular hypertension increases from 2 to 9% over the same time span [7]. POAG is associated with genetic mutations at several loci [8], but the vast majority of POAG patients do not have known genetic mutations. How each of the known genetic mutations leads to POAG is not exactly understood. Consequently, it is a primary neuronal disease without a definable cause [9]. Worldwide, there are approximately 45 million people today with open-angle glaucoma, predicted to rise to 59 million in 2020 [10]. Bilateral blindness is present in 4.5 million people today, predicted to rise to 5.9 million in 2020 [10].

In this article, we focus on biomarkers of POAG in human studies rather than physiological or imaging technologies, in order to further understand the etiological factors in POAG. We include some *in vitro* and animal studies to emphasize the importance of a POAG biomarker. In principle, a biomarker is an indicator of a biochemical feature or facet that can be used to diagnose or monitor the progress of a disease [11]. Recent advances in detection technology have allowed for the identification of numerous biomarkers in POAG [12]. Multiple biomarker panels of common, multifactorial diseases such as cardiovascular [13] and Alzheimer’s disease [14–15] have also been described recently as facilitating the diagnosis and risk management of these diseases. Although the relationship between Alzheimer’s disease and POAG remains obscure, more than 20% of Alzheimer’s patients also have POAG [16]. The plasma concentration of a variety of signaling proteins differs between patients with Alzheimer’s disease and normal control subjects [14], indicating that systemic plasma changes take place along with central nervous dysfunction. Recent studies of cerebrospinal fluid have demonstrated a decrease in β -amyloid, and increases in tau and phosphorylated tau, which form a ‘signature biomarker of Alzheimer’s disease’ [17]. It is likely that cellular insults or molecular defects intersect, leading to neurodegeneration. Similar scenarios may also occur in POAG. To date, POAG does not have a signature profile.

In this article, we have cataloged all known biomarkers in the aqueous humor, TM, optic nerve and blood in patients with POAG. To facilitate comparisons and to offer mechanistic clues, biochemical changes such as up- or downregulation of biomarkers that have been reported in POAG are organized into four categories; namely, ECM, cell signaling molecules, aging/stress and immunity-related changes, which are listed respectively in Table 1 [18–33], Table 2 [34–45], Table 3 [46–59] and Table 4 [60–78].

ECM changes in POAG

The ECM components in the TM are essential for maintenance of the normal aqueous humor outflow [79]. In the TM of POAG eyes, excessive abnormal accumulations, as well as decreases of other ECM materials (Table 1) have been documented [80–81]. The ECM produced by the cells is composed of multidomain macromolecules such as collagens, cell-binding glycoproteins and proteoglycans that link together to form a structurally stable composite. It is now well documented that ECM is a dynamic entity determining and controlling the behavior and biologic characteristics of the cells [82].

One key component of the ECM in the TM is proteoglycans, which are macromolecules consisting of a core protein to which glycosaminoglycan side chains are covalently attached. This class of molecules has been implicated in the maintenance of resistance to aqueous humor outflow ever since Barany demonstrated that perfusion of the anterior chamber with testicular hyaluronidase greatly reduced the outflow resistance in enucleated bovine eyes in the 1950s [79]. In the TM tissue, proteoglycans form gel-like networks that may function as a gel-filtration system. The major types identified include chondroitin, dermatan and heparan sulfate proteoglycans that may represent decorin, biglycan, versican, perlecan and syndecan [82,83].

The relative amounts of each type of glycosaminoglycan in the TM tissue have been determined [20,80]. Hyaluronic acid and chondroitin–dermatan sulfates are the major constituents, with heparan sulfate and keratan sulfate present in smaller amounts. A depletion in hyaluronic acid, and an accumulation of chondroitin sulfates and undigestible glycosaminoglycan material have been associated with POAG conditions [20]. Both chondroitin sulfate and hyaluronic acid have been shown to contribute to flow resistance and influence flow rates *in vitro* [80]. The flow rate was decreased when hyaluronic acid and chondroitin sulfate were used at POAG concentrations. Disrupting glycosaminoglycan chain biosynthesis by sodium chlorate or β -xyloside increases outflow facility in perfusion cultures [81]. Of note, the level of an ectodomain fragment of hyaluronic acid receptor CD44 (sCD44) was found to be elevated in the aqueous humor of POAG patients and the concentration was highly correlated with the clinical visual field loss that characterizes POAG (Table 2) [41]. sCD44 is cytotoxic to TM cells, but the toxicity can be blocked by hyaluronic acid [84]. The decreased hyaluronic acid may thus result in diminished protective capacity and further deterioration in POAG.

Fibronectin, laminin, vitronectin and matricellular proteins that include tenascin and thrombospondin-1 have been localized in the TM [82]. These glycoproteins are crucial in biologic processes such as cell attachment, spreading and cell differentiation [82]. Overexpression of fibronectin, laminin as well as collagen type IV results in a decrease in the TM cell monolayer permeability [85,86]. In addition, the expression of thrombospondin-1 has been shown to be increased in the TM of POAG eyes (Table 1) [28].

Elastin is localized to the central core of sheath-derived plaques or elastic-like fibers in the TM [85]. Fibrillin-1, a component of microfibrils, is found in both the core and the surrounding sheath of the elastic-like fibers. Fibrillin-1 and type VI collagen are also

constituents of long-spacing collagens in the TM [85,86]. It is believed that the collagen fibers and elastic-like fibers are organized in the TM to accommodate resilience and tensile strength, providing a mechanism for reversible deformation in response to cyclic hydrodynamic loading [83]. In trabecular lamellae and in juxtacanalicular connective tissue regions, accumulation of long-spacing collagens and sheath-derived plaques has been documented in POAG and aged eyes [85,87]. The cochlin deposits in the glaucomatous TM (Table 1) appear to increase with age, and are associated with proteoglycans. Such deposits have been proposed to contribute to the increase of ECM resistance to outflow and the POAG pathology [15].

The ECM is constantly modified by the surrounding cells through enzymes such as matrix metalloproteinase (MMP) family members and inhibitors such as tissue inhibitors for MMP (TIMPs) found in the TM [83]. Ongoing ECM turnover, initiated by MMPs, appears to be essential for maintenance of the aqueous outflow homeostasis. MMP-3 and possibly also MMP-9 may be responsible for the efficacy of laser trabeculoplasty; a clinically useful alternative treatment to reduce IOP in patients with glaucoma [83,85]. The addition or induction of MMP-3 in perfused human anterior segment organ cultures increases, whereas blocking the endogenous activity of the MMPs in the TM reduces the aqueous humor outflow facility [83].

The ECM in the TM may also be remodeled in response to exogenous stimuli such as glucocorticoids and oxidative stress [82,85]. Mechanical stretch caused an increase in MMP-1 and MMP-3 activities and alteration of ECM molecules, including proteoglycans and matricellular proteins [88]. The ECM is modulated by cytokines. The most studied cytokine in the TM is TGF- β . A higher than normal level of TGF- β 2 was found in the aqueous humor of patients with POAG (Table 4) [73]. TGF- β 2 upregulated several ECM-related genes in TM cell cultures [89]. In TGF- β 2-perfused organ cultures, focal accumulation of fine fibrillar extracellular material was observed in TM tissues. Furthermore, TGF- β 2 perfusion reduced outflow facility and elevated IOP [90], and adenoviral gene transfer of active TGF- β 2 elevated IOP and reduced outflow facility in rodent eyes [91]. These results suggest that the increased TGF- β 2 level in the aqueous humor may be related to the pathogenesis of glaucoma. Other cytokines such as TNF- α , which is increased in the optic nerve head of POAG (Table 4), also modulate the ECM, probably via regulation of MMPs and TIMPs [82,92].

Signaling molecules in POAG

The TM and optic nerve utilize local and probably systemic cell signaling pathways to maintain cell viability. Locally in the TM, the Rho family of small GTPases has been shown to be of vital importance in the outflow system [93–95]. In the active GTP-bound state, Rho GTPases interact with and activate downstream effectors such as Rho kinase to modulate the assembly of actin structures. In TM cells, a decrease in actin stress fibers and focal adhesions has been shown to occur with treatment of Rho kinase inhibitors, gene transfer of dominant-negative RhoA, and dominant-negative Rho-binding domain of Rho kinase [95]. These cellular changes are associated with reduced myosin light chain phosphorylation and/or enhanced outflow facility. Conversely, molecules including sphingosine-1-phosphate

and endothelin-1 that activate the Rho/Rho kinase signaling pathway through G-protein-coupled receptors promote myosin light chain phosphorylation, and in turn decrease the aqueous humor outflow facility [93–95]. Endothelin-1 has been reported to be increased in the aqueous humor [36] and blood [37] of POAG patients (Table 2).

The aqueous humor also modulates TM cell signaling. It contains albumin as a major constituent. Other components include hydrogen peroxide (H₂O₂), ascorbic acid, cytokines such as TGF- β , HGF and VEGF, and molecules including MMPs, proteinase inhibitors, sCD44 and hyaluronic acid [82]. In both POAG aqueous humor and Alzheimer's cerebrospinal fluid [96], HGF [38] and VEGF [44] increase, indicating modulation of these fluids in the disease process. Since TM cells are in constant contact with the aqueous humor, it is expected that altered levels and/or activities of aqueous humor components would have an impact on the behavior and activities of these cells. The addition of normal aqueous humor rather than the standard fetal bovine serum to monolayers of TM cultures decreases cell proliferation, and produces changes in cellular and molecular characteristics to mimic more closely the TM physiologic profiles *in situ* [97].

Increased levels of angiopoietin-like 7, erythropoietin, plasminogen activator inhibitor, sCD44, phospholipase 2, thymulin (Table 2), glutathione, ascorbic acid (Table 3) and TGF- β 2 (Table 4), but, significantly, a decreased level of hyaluronic acid (Table 1), have been reported in the aqueous humor of POAG eyes. Of note, the decrease in aqueous hyaluronic acid concentration parallels that in the TM, and the increase in sCD44 (the ectodomain portion of the CD44 receptor) also parallels the CD44 receptor increase in the TM (Table 1). Only sCD44 has been tested in rodents; adenoviral gene transfer of sCD44 resulted in a sustained increase in IOP in mice [98]. The sCD44 found in the POAG aqueous humor is hypophosphorylated [99]. The hypophosphorylated form has high cytotoxicity and low hyaluronic acid-binding affinity, and is suggested to represent a pathophysiologic feature of the disease process [99].

Stress & aging in POAG

Trabecular meshwork cellularity is reduced with aging [100,101]. Morphologic studies have also revealed thickened basement membranes and an accumulation of sheath-derived plaques and long spacing collagens in the TM of aged eyes [90]. The number of senescent cells that stain positive for senescence-associated β -galactosidase is increased in the TM of POAG eyes (Table 3) [57], further supporting the hypothesis that POAG is an age-related disease [82,102]. Oxidative damage and stress have also been implicated as contributing to the morphologic and physiologic alterations in the aqueous outflow pathway in aging and glaucoma (Table 3) [103]. The TM is known to be exposed to 20–30 μ M H₂O₂ present in the aqueous humor and is subjected to chronic oxidative stress [85]. Superoxide dismutase, an enzyme involved in the protection against oxidative damage, has been shown to decline with age in human TM tissues [102]. TM cells also synthesized a specific set of proteins, such as α B-crystalline, that may act as molecular chaperones to prevent oxidative or heat shock damage [45]. Markers of oxidative damage [103,104], abnormalities in mitochondrial DNA [105] and diminished blood levels of oxidant scavengers glutathione [53] are found in POAG patients. It appears that oxidative stress that exceeds the capacity of TM cells for

detoxification is involved in damaging the cells and alteration of the aqueous humor outflow, leading to increased IOP. Moreover, stress/aging-related changes also occur in the red blood cell phospholipid composition, which may alter red blood cell flexibility in POAG, causing reduced ocular blood flow and optic nerve axon loss [52]. Linear regression analysis suggests that a decrease in phosphatidylcholine-carrying docosahexaenoic acid (an ω -3 fatty acid) occurs years before the onset of POAG. This underscores the notion that aging, and specifically membrane lipid composition, is a risk factor in POAG [52]. In a longitudinal study, a decrease in phospholipid composition correlates with the development of Alzheimer's disease [106]. There is, however, a possible correction for the change in lipid composition of membranes. One effect of dietary ω -3 is the downregulation of Toll-like receptor (TLR)4 innate immune receptors [107], whereas supplement-free fatty acids upregulate TLR4 [108].

Innate & adaptive immunity in POAG

The immune system is actively involved in the POAG disease process. Innate immunity is the first line of defense and is rapidly activated by trauma and infection (Figure 2) leading to activation of NF- κ B responsive genes [109]. In Figure 2 we hypothesize possible scenarios involved in the POAG disease process. TLR4 recognizes a variety of pathogen-associated molecular patterns, including Gram-negative bacterial lipopolysaccharide (LPS), host heat-shock proteins (HSPs), low-molecular-weight hyaluronic acid fragments and many others [109]. Although low-molecular-weight but not high-molecular-weight hyaluronic acid is known to be proinflammatory [110], the discovery of low-molecular-weight hyaluronic acid signaling through CD44–TLR4–MD2 suggests a unifying cell signaling pathway heretofore not appreciated in the inflammatory cascade. In addition, mutations in two genes encoding myocilin and optineurin may signal as negative suppressors in the signaling pathway shown in Figure 2. Optineurin negatively regulates IFN- β by interacting with TBK1 [111]. Myocilin, a member of the olfactomedin domain family, may act in a similar way as olfactomedin 4, by downregulating NF- κ B response to *Helicobacter pylori* infection [112]. These observations of low-molecular-weight hyaluronic acid, optineurin and myocilin fitting into a possible unified signaling pathway leading to the inflammatory cascade are novel, and demonstrate the many ways in which POAG may occur.

Toll-like receptor 2 recognizes bacterial peptidoglycan lipoprotein, porins, viral hemagglutinin and serum amyloid A [113]. Increases in a number of TLRs in the retina of POAG patients is remarkable (Table 4). Upregulation of acute stress response protein amyloid in the TM of POAG patients further supports the notion that POAG has ocular altered protein expression [67]. Treatment of cultured human TM cells with recombinant serum amyloid A affected gene expression, including a 22-fold upregulation of IL-8 [67]. TLRs are expressed on monocytes, dendritic cells, neutrophils, mucosal epithelial cells and endothelial cells [114]. Some TLRs are expressed on the surface of cells, whereas others are expressed in the cell compartment [114]. Heat-shock proteins are known to activate the innate immune system [115]. Notably, HSP-27 and HSP-60 immunization in rodents caused retinal ganglion cell neuron loss similar to that observed in POAG [116]. Thus, several TLRs and HSPs are upregulated, indicating optic nerve involvement in the innate immune response in POAG.

The adaptive immune system is the second line of defense, and relies on antigenpresenting cells required for lymphocyte activation and effector cells that eliminate antigens [114]. Cytokines are secreted by cells of the innate and adaptive immune system in response to a variety of inflammatory and antigenic stimuli [117]. Excessive amounts or actions of cytokines can lead to pathological consequences. In both POAG aqueous humor [70,71] and Alzheimer's cerebrospinal fluid [118], IL-8 is increased. See Table 4 for changes in the concentrations of cytokines in the aqueous humor, optic nerve and blood of POAG patients. It is of note that Alvarado *et al.* have shown that conditioned media obtained from irradiated TM and cytokines modulated aqueous outflow *in vitro* [119,120]. In addition, autoantibodies are also present in the blood of POAG patients (Table 4).

Conclusion & perspectives

This article summarizes the current knowledge of possible biomarkers in POAG. The exact role of each biomarker is sketchy at present, and a direct link to POAG remains to be established. While individual biomarkers are only indicative of POAG, combining multiple biomarkers for risk assessment in POAG is a goal for the future. The regulation of TGF- β , CD44, Wnt signaling [121] and innate immunity cascades [122] are all areas of active research. Innate immunity could be modulated by supplemental dietary ω -3 fatty acids because they enhance the recruitment of TLR4 into caveolae [123]. Two key components of lipid rafts with caveolae, caveolin-1 and caveolin-2, were recently identified by a genome-wide association study for POAG as possible risk factors for the disease [124]. The observation of downregulation of lipids in red blood cell membranes in both POAG and Alzheimer's disease, which precede the development of the diseases coupled with modulation of TLR4 by ω -3, is intriguing. Moreover, the recent identification of caveolin-1 and caveolin-2 as a risk factor [124] underscores the importance of hyaluronic acid, ω -3 fatty acids and TLR4 in the inflammatory cascade leading to the POAG disease process. An emerging theme perhaps applicable to both POAG and other neurodegenerative diseases is that selective cell vulnerability occurs as a result of dysfunctional pathways, stress, aging or other insults, and may eventually lead to the disease process. Biochemical changes listed in Tables 1–4 indicate that diverse pathways/mechanisms/molecules including proinflammatory cytokines may trigger dysregulation of normal defense mechanisms, faulty signaling and/or progressive fibrosis leading to the disease process. A deeper understanding of the various mechanisms is a prerequisite for the design of novel gene and immune therapies, or other treatment modalities for POAG.

Expert commentary

In searching the current biomarkers of POAG, 67 biomolecules have been found in increased or decreased concentrations in the ECM, cell signaling, stress and aging, and innate and adaptive immunity. Notably, the majority were increased and a few decreased in POAG. These observations may reflect alterations contributing to the clinical disease progression, or simply epiphenomenons. Missing in the current status is the initiator, the prime cause of the disease. Genetic mutations have yielded clues but no answer is known to date as to the cause of the disease. Our current status is therefore innumerable biomarkers, which clearly show notable changes in ocular tissues, that is, the TM and optic nerve as well

as systemically in blood elements and the immune system. Thus, determining the cause of POAG remains a challenge for future research.

Five-year view

In POAG, the words ‘the search goes on’ echo in basic science researchers and ophthalmologists trying to find the answers to the causes of this neurodegenerative disease. Current attempts to solve the disease have focused on two strategic arenas: the TM function and its impact on IOP as a major risk factor for the disease, and the optic nerve dysfunction leading to retinal ganglion axonal loss and visual impairment. Genetic mutations have yielded clues as to the cause of POAG, but as yet many questions remain unanswered. For example, mutations in myocilin and optineurin genes are connected to the phenotype, but how would they trigger the disease? In the next 5 years, priority areas of research that include signaling pathway discoveries, multiplex biomarker panels and continued genomic studies are expected to elucidate the common final pathways of the disease. Certainly, the final pathways are complex and redundant such that the overlap of bioinformatics will be challenging. Current studies suggest that altered TGF- β 2 and sCD44 pathways and their interactions with the innate and adaptive immune system are important clues to both TM and optic nerve pathophysiology. When the POAG disease pathways are unraveled and the answers yield the cause, POAG will be renamed open-angle glaucoma, and drug discoveries and new treatment modalities will be available for better regulation of IOP and neuroprotection for the optic nerve.

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Key issues

- Primary open-angle glaucoma (POAG) is characterized by loss of retinal ganglion cells and their axons, resulting in optic nerve cupping and visual field loss.
- POAG is the most common type of glaucoma, the second leading cause of blindness worldwide and the leading cause of blindness in African-Americans.
- POAG has no known cause.
- The major ocular risk factors for POAG include intraocular pressure elevation and aging.
- Biomarkers in the aqueous humor, trabecular meshwork, optic nerve and blood are organized into four categories; namely, extracellular matrix, cell signaling molecules, aging/stress and immunity-related changes.
- In searching for the current biomarkers of POAG, 67 biomarkers have been found in increased or decreased concentrations.
- Biomarkers show notable changes in ocular tissues, that is, the TM and optic nerve, as well as systemically in blood elements and the immune system. Thus, the POAG is an ocular disease with systemic findings without a known cause.
- Priority areas of research are signaling pathway discoveries and multiplex biomarker panels, as well as continued genomic studies that are expected to elucidate the common final pathways of the disease.

Figure 1. Human optic nerve clinical photographs ([A] normal and [C] advanced primary open-angle glaucoma) and postmortem photomicrographs of optic nerve heads ([B] normal and [D] advanced primary open-angle glaucoma; hematoxylin and eosin stain $\times 20$)

In the normal human optic nerve, the physiologic cup is small (*) with a ratio of cup to disc of 0.2, whereas in the advanced primary open-angle glaucoma the cup is much larger with a ratio of cup to disc of 0.8. The increase in the size of the cup is due to loss of nerve fibers and deformation of the lamina cribrosa. Reprinted with permission from [2].

Figure 2. Putative activation of Toll-like receptor 4 occurs by trauma/aging to the extracellular matrix trabecular meshwork or by mechanical deformation, that is, cupping of the optic nerve, leading to breakdown of hyaluronic acid into hyaluronic acid oligosaccharides

Low-molecular-weight hyaluronic acid oligosaccharides (depicted as blue squares) are released from the ECM as a result of trauma and regulated by the balance of hyaluronidase inhibitors and hyaluronidase. These oligosaccharides bind to TLR4 along with coaccessory molecules lymphocyte antigen 96 (MD2) and the CD44 receptor. This receptor complex utilizes ERM proteins and MyD88. Ras and NF-κB are upregulated, which leads to downstream production of effectors including MMP-13, TGF-β2, SOCS3 and inflammatory

cytokines. By contrast, Gram-negative bacteria release membrane fragments containing LPS (depicted as red hexagons). The fragments bind to LBP, which bind to TLR4s that require CD14 and MD2. Ligand binding to TLR4 activates certain protein kinases (e.g., IRAK, TRAFs and TBK1) that amplify the signal, leading to the inflammatory response and its modulation of the ECM, cell signaling molecules, stress response and immunity related changes. The summation of these protein responses, which result in the final common pathway, may lead to phenotype primary open-angle glaucoma. Certainly, the important feature of this figure is the initial activation of the TLR4 and subsequent downstream effects. Shown in this figure are proposed signaling pathways and components based on the work of several groups. Please note that both pathways lead to the activation of NF- κ B, TRIF, TRAM, Mal, ERK, RIP1, IRF-3, JNK and MIP-2. ERM: Ezrin, radixin, moesin; HAS: Hyaluronic acid synthetase; IRF: Interferon-regulatory factor; LBP: Lipopolysaccharide-binding protein; LPS: Lipopolysaccharide; Mal: MyD88 adaptor-like protein; MIP-2: Macrophage inflammatory protein 2; MMP: Matrix metalloproteinase; MyD88: Myeloid differentiation primary response gene 88; Ras: Rat sarcoma G protein; RIP: Receptor-interacting protein; SOCS3: Suppressor of cytokine signaling 3; TLR: Toll-like receptor; TRAM: TRIF-related adaptor molecule; TRIF: Toll/IL-1 receptor-domain-containing adaptor-inducing IFN- β . Reproduced with permission from [109].

Table 1

Extracellular matrix changes in primary open-angle glaucoma.

Biomarker	Aqueous humor	Trabecular meshwork	Optic nerve	Systemic (serum/plasma)	Ref.
<i>ECM elements</i>					
CD44	↓				[18]
Cochlin	↑				[19]
Chondroitin sulfate	↑				[20]
Collagen type IV		NC			[21]
Elastin	↑		↑		[22,23]
Fibronectin	NC	NC			[21,24]
Hyaluronic acid	↓↓	↓↓↓↓	↓		[20,25,26]
GA Gase-resistant material		↑↑↑↑			[20]
Tenascin			↑		[27]
Thrombospondin-1		↑			[28]
<i>ECM remodeling enzymes and inhibitors</i>					
MMP-1		↑	↑		[29,30]
MMP-3	NC	↑	↑		[29–31]
MT1 MMP				↑	[32]
TIMP-1	NC	↑			[29,31]
TIMP-2	↑				[33]

The increase or decrease in the reported changes in primary open-angle glaucoma are expressed as statistically significant ↑ or ↓. Whenever possible, a twofold change is denoted by two arrows, a threefold change by three arrows and a fourfold change by four arrows.

ECM: Extracellular matrix; GA Gase: Glycosaminoglycan-degrading enzyme; MMP: Matrix metalloproteinase; MT1: Membrane type 1; NC: No change; TIMP: Tissue inhibitor for matrix metalloproteinase.

Table 2

Cell signaling changes in primary open-angle glaucoma.

Biomarker	Aqueous humor	Trabecular meshwork	Optic nerve	Systemic (serum/plasma)	Ref.
Angiopoietin-like 7	↑↑↑				[34]
Erythropoietin	↑				[35]
Endothelin-1	↑			↑	[36,37]
HGF	↑				[38]
Phospholipase A ₂	↑				[39]
Plasminogen activator inhibitor-1	↑↑↑				[40]
Soluble CD44	↑↑				[41,42]
Thymulin				↑↑↑	[43]
VEGF	↑			↑	[44,45]

The increase or decrease in the reported changes in primary open-angle glaucoma are expressed as statistically significant ↑ or ↓. Whenever possible, a twofold change is denoted by two arrows and a threefold change by three arrows.

Table 3

Stress and aging changes in primary open-angle glaucoma.

Biomaker	Aqueous humor	Trabecular meshwork	Optic nerve/retina	Systemic (serum/plasma/red blood cells)	Ref.
Acetylcholinesterase				↑	[46]
3- α -hydroxysteroid dehydrogenase				↓	[47]
Ascorbic acid	↑↑↑				[48]
Cortisol				↑	[49]
ELAM-1		↑			[50]
Fatty acid: – Eicosapentenoic acid – Choline plasmalogens – Docosahexaenoic acid				↓ ↓ ↓	[51] [52] [52]
Ω -3				↓	[52]
Glutathione	↑↑↑			↓	[53,54]
Nitric oxide	↑				[55]
Nitric oxide synthase				↑	[56]
NF- κ B		↑			[57]
HIF-1 α			↑		[58]
Senescence-associated β -galactosidase		↑			[59]

The increase or decrease in the reported changes in primary open-angle glaucoma is expressed as statistically significant ↑ or ↓. Whenever possible, a twofold change is denoted by two arrows and a threefold change by three arrows.

ELAM: Endothelial cell leukocyte adhesion molecule; HIF: Hypoxia-inducible factor.

Table 4

Innate and adaptive immunity changes in primary open-angle glaucoma.

Biomaker	Aqueous humor	Trabecular meshwork	Optic nerve	Systemic (serum/plasma)	Ref.
<i>Innate immunity</i>					
<i>Receptors</i>					
TLR2			↑↑		[60]
TLR4			↑↑↑		[60]
TLR7			↑		[60]
TLR8			↑		[60]
<i>Associated proteins</i>					
MyD88			↑		[60]
CD44		↑			[18]
<i>Ligands (pathogen-associated molecular patterns)</i>					
HSP-60 (bacterial)			↑		[61,62,64]
<i>Helicobacter pylori</i>	↑↑↑				[63]
<i>Heat-shock proteins</i>					
HSP-27			↑↑↑↑	↑	[65,66]
αβ crystallin			↑↑↑	↑	[60,62,66]
HSP-60			↑↑↑↑	↑	[60,66]
HSP-70			↑↑↑↑	↑	[60,66]
Serum amyloid A		↑↑			[67]
<i>Adaptive immunity</i>					
<i>Cytokines</i>					
IL-2				↑	[68]
IL-4				↑↑	[69]
IL-8				↑↑	[69,70,71]
IL-12				↑↑	[69]
TGF-β2	↑				[72,73]
TNF-α	NC		↑	↓	[30,69,74]

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Biomaker	Aqueous humor	Trabecular meshwork	Optic nerve	Systemic (serum/plasma)	Ref.
<i>Autoantibodies</i>					
Fodrin			↑		[75]
Gluthathione S-transferase			↑		[76]
Neuron-specific enolase			↑		[77]
Retinal S-antigen			↑		[78]
Vimentin			↑		[66]
Monoclonal gammopathy			↑		[76]

The increase or decrease in the reported changes in primary open-angle glaucoma are expressed as statistically significant ↑ or ↓. Whenever possible, a twofold change is denoted by two arrows, a threefold change by three arrows and a fourfold change by four arrows.
HSP: Heat-shock protein; MyD88: Myeloid differentiation primary response gene 88; NC: No change; TLR: Toll-like receptor.