REVIEW

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The impact of matrix metalloproteinases and their tissue inhibitors in patients with chronic glaucoma – a literature review

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Abstract

Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) play an important role in the pathophysiology of chronic glaucoma, as they are involved in extracellular matrix (ECM) remodeling in the trabecular meshwork (TM), affecting its ability to efficiently regulate intraocular pressure (IOP). Ensuring the balance between MMPs and TIMPs helps to maintain homeostasis in ocular tissues, which is essential to avoid glaucomatous lesions. Elevated levels of MMPs and increased degradation of the ECM, ultimately affecting aqueous humor outflow and increasing IOP, characterize glaucoma. In the current literature review, the impact and interactions of MMPs and TIMPs in chronic glaucoma have been emphasized, with multiple but still unelucidated roles in the mentioned pathology including their clinical implications, future research directions, and therapeutic approaches. Research to date indicates that the expression of TIMPs is altered in patients with chronic glaucoma, suggesting a compensatory response to increased MMPs activity. Certain drugs can influence the expression levels of MMPs and TIMPs, therefore therapeutic strategies can be developed to restore the balance between tissue enzymes and their inhibitors. Therefore, understanding the relationship between MMPs and TIMPs is a key factor in the pathogenesis of chronic glaucoma. Understanding the interplay between the two provides interesting insights into ECM remodeling in ocular tissues, highlighting the potential of targeted therapies to restore the balance between proteolytic enzymes and their inhibitors.

Keywords: zinc-dependent proteolytic enzymes, inhibitors of proteolytic enzymes, primary open-angle glaucoma, primary angle-closure glaucoma.

Introduction

Chronic glaucoma is a progressive neurodegenerative disease that occurs due to the degeneration of retinal ganglion cells (RGCs) and the optic nerve, ultimately leading to permanent and irreversible vision loss. Although the most significant risk factor in disease progression is elevated intraocular pressure (IOP), among others, however, the pathophysiology of the disease remains complex and unelucidated [1, 2]. The gradual increase in IOP usually presents asymptomatically until a significant visual loss occurs [3, 4]. Understanding the risk factors that contribute to the progression of glaucoma is very important for early detection and effective management, as well as for improving the quality of life of affected patients. Increased IOP contributes to the pathogenesis of glaucoma, and it is considered one of the modifiable risk factors for its progression [5–7]. Management of elevated IOP values is a key factor to consider in glaucoma treatment strategies, as these elevated values can affect the optic nerve by disrupting ocular blood flow [5, 6]. A non-modifiable risk factor for disease progression is age, with a significant prevalence of glaucoma being observed in elderly patients [1]. It has also been observed that genetic predisposition for glaucoma development is another factor to be considered, with a family history of glaucoma being associated with an increased risk of developing the disease. In addition, a pattern of glaucoma development has been observed among black populations, again with genetics playing a key role [1, 8]. A controversial issue in glaucoma research is the impact of other systemic pathologies, such as hypertension. A worrying association has been observed between chronic hypertension and glaucoma, with hypertensive patients showing exacerbations of glaucomatous lesions as well as impaired ocular blood flow. Furthermore, it has been observed that lifestyle factors may have implications for pathogenesis of glaucoma, with smoking and alcohol, for instance, being correlated with an increased risk of glaucoma development and worsening. Studies have shown that smokers maintain a continuous inflammatory process in the retinal cells, often accompanied by retinal cell apoptosis, leading to a poor prognosis and progression of glaucoma [9, 10]. Surprisingly, another risk factor identified for the development of glaucoma is obstructive sleep apnea, which

is correlated with the occurrence of ocular vascular and metabolic disorders [11]. Among the ocular factors most often used to assess the individual susceptibility of patients to develop glaucoma are central corneal thickness and cup-to-disc ratio (CDR) [12]. Both the determination of central corneal thickness and the CDR, as well as IOP measurements are very important parameters in patients with ocular hypertension because they have a diagnostic role in estimating the risk of glaucoma progression in this category of patients [13].

In terms of classification, chronic glaucoma can be divided into two categories, these being the most common forms: open-angle glaucoma (OAG) and angle-closure glaucoma (ACG). Worldwide, a higher prevalence of OAG has been observed [3, 4, 14]. Regarding ACG, it can be further subdivided into two categories: acute ACG and chronic ACG. It has been observed that chronic ACG occurs as a result of gradual closure of the anterior chamber angle. This further leads to increased IOP and, over time, progressive optic nerve damage [15].

The two types of glaucoma, OAG and ACG, belong to the category of primary glaucoma and occur without any identifiable cause, while secondary types of glaucoma develop due to other ocular or systemic conditions [16]. For example, neovascular glaucoma – a type of secondary glaucoma, is often associated with chronic diseases (diabetes), leading to significant visual impairment if not properly diagnosed and treated [17].

Epidemiologically, one of the leading causes of irreversible blindness worldwide is chronic glaucoma, with millions of people affected, especially in the elderly population [18, 19]. In some regions, the prevalence of OAG is particularly high, affecting about 2.53% of the population [20, 21]. Due to the treacherous nature of the disease, chronic glaucoma is often diagnosed late, therefore, regular screening of patients and increased awareness are necessary measures in early detection of this disease [22, 23].

Several mechanisms underlie the development of glaucoma, including vascular dysregulation, neuroinflammation, and oxidative stress, contributing to RGC degeneration and optic nerve damage. Increased IOP induces mechanical stress on the optic nerve head, leading to axonal damage and RGC apoptosis [24, 25]. This mechanism triggers numerous distinct molecular cascades, including the activation of apoptotic pathways and the involvement of neuroinflammatory processes [26, 27]. Furthermore, in the study developed by Kim *et al.* [28] it was shown that chronic ocular hypertension leads to morphological changes in RGCs, including dendritic atrophy and axonal degeneration, early indicators of glaucomatous lesion. In addition, factors such as metabolic syndrome, which is associated with insulin resistance and chronic low-grade chronic inflammation, have been identified as independent risk factors in the development of glaucoma [29].

Another key mechanism in glaucoma progression is neuroinflammation. Bosco *et al.* have shown that activation of microglia, *i.e.*, resident immune cells of the central nervous system, contributes to RGC degeneration by releasing proinflammatory cytokines and reactive oxygen species (ROS) [30]. This inflammatory response exacerbates oxidative stress, further damaging RGCs and promoting their apoptosis [31]. The neurodegenerative aspect of glaucoma is supported by findings suggesting pathophysiological features in common with other neurodegenerative diseases, such as Alzheimer's disease (AD), emphasizing the role of neuroinflammation and RGC death [32]. Another identified mechanism underlying the pathophysiology of glaucoma is oxidative stress, which affects RGCs, damaging them and, over time, leading to their apoptosis. Underlying the pathophysiology of glaucoma *via* oxidative stress is glutamate-induced excitotoxicity in the eye [33]. The progressive damage and loss of RGCs are modulated by the impact of mechanical stress, neuroinflammation, and oxidative damage on ocular health. The oxidative stress can lead to dysfunction of aqueous humor drainage in glaucoma patients, exacerbating the increase in IOP, thus leading to a worsening of the disease [25].

Vascular factors, such as the potent vasoconstrictor endothelin-1, have been observed to have implications for the pathophysiology of glaucoma, and have been correlated with glaucoma progression. The mechanism underlying this process is their ability to induce ischemia in the retina, affecting the survivability of RGCs [26]. Furthermore, the progression of glaucomatous damage has been shown to occur as a result of impaired blood flow function, thus affecting the oxygen supply to the optic nerve [31].

Studies in animal models confirm changes observed in glaucoma patients with chronic ocular hypertension, including progressive outer retinal degradation, optic nerve damage, or loss of RGCs [34]. A common problem among patients in the effective management of chronic glaucoma is patient non-adherence to treatment regimens. This non-adherence may be caused due to a lack of understanding of the chronicity and severity of the disease, lack of communication with specialized medical staff, or socio-economic problems. All these causes can have a negative impact on the management of chronic glaucoma, leading to the worsening of the disease and, gradually, to irreversible vision loss due to a lack of effective approaches [35]. Therefore, improving patient and caregiver education is the key to improving treatment adherence and ultimately preserving vision in people with chronic glaucoma.

Overview of matrix metalloproteinases

Definition, function, and classification of MMPs

Matrix metalloproteinases (MMPs) are defined as a family of zinc-dependent endopeptidases with a major role in extracellular matrix (ECM) remodeling. These enzymes contribute to the degradation of different components of the ECM, facilitating certain tissue processes (repair, development, and remodeling) in response to pathological and physiological stimuli [36]. MMPs are secreted as inactive proenzymes, requiring proteolytic activation to become functional. Concerning the regulatory mechanisms of MMPs, their activation is mediated by serine proteases or other types of MMPs. It has been observed that it is more important to consider the functionality of MMPs than ECM degradation. This is supported by the important roles that MMPs play in the modulation of cell signaling pathways and inflammatory responses by acting on bioactive molecules involved in these pathways, such as cytokines or growth factors [36, 37]. Research over the years has shown that MMP-9, for example, promotes the activation

of transforming growth factor-beta (TGF-β), which plays an important role in inflammatory cell processes and fibrotic processes [38]. Based on these considerations, MMP-9 has an important role in various pathological processes such as cancer metastasis, chronic inflammatory disorders, or cardiovascular diseases [39]. MMPs can be grouped both according to their structure and according to the specificity of the substrate on which they act. The main groups of MMPs include collagenases, stromelysins, gelatinases, and membrane-type MMPs (MT-MMPs) [40]. It is important to know the groups to which each MMP belongs because based on their classification, the biological mechanisms underlying each class and the various implications in pathophysiological processes can be understood. The most outstanding examples of the gelatinase class are MMP-9 and MMP-2, the metalloproteinases of this class having a common mechanism of action for the degradation of type IV and type V collagen. An example of the relevance of the knowledge of the biological mechanisms and particularities of each MMP subtype is supported by the study of Sanii *et al.* [41], who showed that MMP-2 of the gelatinase class might be useful in oncology, serving as an important biomarker, as it can distinguish between benign and malignant thyroid neoplasms. van Haaften *et al.* [42] stated that MMP-9 has been implicated in inflammatory disorders (*e.g.*, Crohn's disease), where its expression correlates with both disease activity and tissue remodeling. MT-MMPs, including MT1-MMPs, are very important in pericellular proteolysis and cell signaling, essential in both tumor progression and metastasis [43]. For example, Yao *et al.* [40], demonstrated that MT1-MMP activates pro-MMP-2, resulting in enhanced invasive capabilities of cancer cells. This activation is often regulated by diverse signaling pathways, including those mediated by mitogen-activated protein kinases (MAPKs), which influence MMP expression in response to extracellular stimuli.

In addition to their role in cancer, MMPs are also involved in the immune response and infectious diseases. This role facilitates the recruitment of leukocytes and modulates the activity of cytokines and chemokines, two immunomodulatory agents vital for host defense mechanisms. However, excessive MMP activity can lead to tissue damage and contribute to the pathogenesis of various diseases, including chronic inflammatory conditions and cancer [44].

Tissue inhibitors of metalloproteinases (TIMPs) bind to MMPs inhibiting their enzymatic activity and regulating it, thus leading to the maintenance of ECM homeostasis and prevention of pathological remodeling. Loss of control of TIMPs over MMP activity leads to an imbalance between the two, and further to pathological conditions such as fibrosis, where excessive ECM deposition occurs, or cancer, where increased MMP activity leads to tumor invasion and metastasis [45].

Role of MMPs in chronic glaucoma

The role of MMPs in the pathophysiology of chronic glaucoma is significant, in particular, due to their involvement in ECM remodeling in ocular tissues. It is known that MMPs, including MMP-2 and MMP-9, degrade various components of the ECM, thereby influencing IOP, and thereby contributing to glaucomatous damage. Elevated levels of MMP-2 and MMP-9 are associated with increased ECM remodeling in the conjunctival stroma of glaucoma patients compared to healthy patients, indicating that these enzymes may facilitate pathological changes in ocular tissues [46]. Similarly, MMP-9 has been identified as a critical factor in the degradation of ECM components leading to neuronal degeneration and RGC apoptosis. In terms of the mechanisms underlying the pathogenesis of glaucoma, an unfavorable correlation between the disease and elevated MMP levels has been demonstrated. Elevated MMP levels disrupt the balance between ECM synthesis and degradation. This disruption results in decreased resistance to aqueous humor leakage through the trabecular meshwork (TM) [47, 48]. For example, increased levels of MMP-9 expression have been identified in the aqueous humor of glaucoma patients. Based on these considerations, MMPs serve as biomarkers for glaucoma progression, targeting them being a key aspect of therapeutic intervention in glaucoma [49, 50]. A worrying correlation between MMP, ECM, and inflammatory processes in glaucoma has been identified, as this interaction may lead to a worsening and exacerbation of glaucomatous lesions. This is due to the continued maintenance of the inflammatory process due to the stimulation of proinflammatory cytokines by MMP, leading to a marked degradation of the ECM [51].

Another important aspect underlying the pathogenesis of glaucoma is the interaction between MMPs and TIMPs. It has been observed that an imbalance in the activity of MMPs can lead to prolonged inflammation and pathological tissue remodeling, both of which lead to a poor prognosis and progression and worsening of glaucoma [52]. Genetic studies have identified a polymorphism in the *MMP-9* gene, indicating that genetic predisposition negatively influences both the expression and activity of MMPs in patients with primary glaucoma [48].

Tissue inhibitors of metalloproteinases

Structure and function

There is an important interaction between TIMPs and the ECM, since TIMPs play a role in ECM regulatory processes, largely by inhibiting MMP activity. Regarding the categorization of TIMPs, there are four types: TIMP-1, TIMP-2, TIMP-3, and TIMP-4, with distinct roles in regulatory and physiological processes as well as pathological conditions. For example, TIMPs are involved in inflammatory processes, tissue remodeling, or cancer cell invasion [53]. The basic structure of each type of TIMP consists of two domains: the N-terminal domain and the C-terminal domain. The N-terminal domain functions to interact with the catalytic domain of MMPs, while the C-terminal domain plays a role in cell signaling processes [54]. However, there is also structural diversity between each type of TIMP, which allows for differential regulation to modulate the activity of MMPs. This differential regulation has an important role in essential biological processes for the organism such as tissue repair and tissue remodeling. A good example of this differential regulation can be given by the unique binding affinity and specificity towards MMPs of TIMP-1 and TIMP-2 members, which has a different influence on tumor progression or tissue regeneration [55]. Recent studies have demonstrated differential binding mechanisms of TIMPs

based on the elucidation of their three-dimensional structures. An example in this regard of understanding how TIMPs inhibit MMPs would be the elucidation of the crystal structure of the TIMP-1/MMP-1 complex, which provided insight into the interaction interface [56]. It was also found that the member TIMP-3 has an affinity for glycosaminoglycans and binds to glycosaminoglycans in the ECM, playing a role in both modulating ECM interactions as well as inhibiting MMPs [57].

TIMPs have several functions in the body, the main function being the inhibition of MMPs activity to regulate ECM progression and maintain tissue integrity, but other important functions may involve tissue homeostasis and cell signaling pathways [58]. For example, the study by Suryapranata *et al.* demonstrated that TIMP-1 can form a stoichiometric complex with MMP [59]. This complex can effectively inhibit the proteolytic activity of MMP and may have implications in promoting cell survival and proliferation, this being independent of its inhibitory effects on MMP. The formation of this complex is particularly significant in the context of cancer, as the TIMP/MMP balance influences tumor cell invasion and metastasis. Increased levels of TIMP-1 are associated with a poor prognosis in colorectal cancer, and TIMP-1 may be considered a potential biomarker in cancer [59]. Also, similarly, expression of the member TIMP-3 has been correlated with favorable outcomes in hepatocellular carcinoma, as it is a tumor suppressor [60]. Paradoxical effects have also been observed for TIMPs, as they may inhibit tumor invasion by acting on limiting ECM degradation, but when overexpressed, tumor cell growth and metastasis may be stimulated, leading to increased angiogenesis and tumor cell survival [61]. For example, it has been observed that TIMPs may contribute to a tumor-promoting microenvironment, with elevated levels being associated with poor prognosis in cancer patients [59, 61]. The member TIMP-3 has also been shown to have implications in osteoarthritis (OA) and other degenerative diseases due to its ability to significantly inhibit aggrecanases involved in cartilage degradation [62].

Because they have dual functionality manifested by inhibition of ECM degradation and promotion of cellular processes that can lead to tumorigenesis, the roles of TIMPs can be complicated and complex in various pathologies. It has been identified that in addition to correlating with MMPs inhibition, TIMPs play an important role in certain signaling pathways due to the C-terminal domain in the structure, these pathways being associated with cell proliferation and tumor cell survival in different types of cancer. An example regarding the activity of TIMPs in activating signaling pathways can be represented by the activity exerted by TIMP-2 type on the stimulation of cell growth and migration concomitant with the activation of c-Src or focal adhesion kinase (FAK) [63].

In current research on the pathophysiological processes underlying certain pathologies, the targeted activity and interaction between TIMPs and MMPs are increasingly being investigated. In the study performed by Thiele *et al.* [64], it was shown that in liver fibrosis, modulations on TIMP may be favorable, as it helps both to restore ECM balance and to decrease fibrogenesis. Another area of interest in the current dynamics in the field is the discovery and testing of therapeutic agents with inhibitory potential on MMP activity. These attempts aim to establish a balance by reducing the ratio of TIMPs to MMPs in pathologies. An example of agents exhibiting these properties is the tetracycline class [65]. It is important to follow targeted therapies in diseases associated with ECM dysregulation due to disturbance of the TIMP/MMP balance, such as cancers or fibrosis. For example, research by Stetler-Stevenson & Gavil [61] has shown that by targeting TIMP-2, it was possible to normalize the tumor microenvironment and improve disease prognosis. A new research direction in this area would be to uncover the roles and implications of each type of TIMP in a wider range of tissues, to improve disease prognosis and achieve more targeted and effective treatment interventions.

Correlation of TIMPs with ocular health and disease

Another important aspect of TIMPs is the discovery of the roles they play both in the maintenance of ocular health and in the development of pathophysiological processes involving the eye. It is well known that there must be a balance between the activity of TIMPs and that of MMPs, as this is how ocular health and homeostasis of ocular tissues are maintained. If this balance is disturbed, there is a risk of various ocular pathologies of varying severity, such as dry eye, glaucoma, or scleritis. It has been stated that an increased level of MMP-9 leads to ocular surface damage and inflammation, thus, MMPs could be considered biomarkers for assessing the severity of dry eye disease [66]. Furthermore, it has been observed that hyperosmolarity leads to triggering the secretion of pro-inflammatory mediators and MMPs, which may exacerbate the chronic inflammation characteristic of dry eye disease, thus leading to further disruption of the tear film, contributing to ocular surface damage [67]. Another pathology that involves the regulatory role of TIMPs is scleritis, in which tissue damage attributed to MMP production is observed, which can lead to significant damage to scleral and episcleral tissues [68]. A balance between MMP and TIMP is required to maintain the structural integrity of ocular tissues and to modulate ocular inflammatory responses [69].

The study developed by Fountoulakis *et al.* demonstrated that the levels of TIMP-1 identified in aqueous humor show changes in patients suffering from OAG, suggesting a correlation between TIMP deregulation and the pathogenesis of ocular diseases such as glaucoma [70]. The role that TIMPs play in chronic glaucoma is increasingly recognized, mainly due to their ability to regulate MMPs, with implications in the remodeling of ECM dynamics in the TM and optic nerve head, thus a balance between MMPs and TIMPs is necessary to avoid disruption of the normal ECM change [71, 72]. In glaucoma, it has been shown that an imbalance between TIMPs and MMPs affects aqueous humor outflow and IOP, leading to TM remodeling. Also, the involvement of TIMPs in ocular health is supported by studies such as the one by Chen *et al.* emphasizing that upregulation of TIMPs is necessary to combat elevated MMPs levels in patients with acute primary angle closure and those with cataracts [73]. In addition, the study by Maddineni *et al.* demonstrates an upregulation of MMPs (especially MMP-2 and MMP-9) in glaucomatous tissues, leading to a

deregulation of the ECM, accompanied by excessive remodeling. As a consequence of these dysregulations, thickening of the TM as well as a decrease in the intertrabecular spaces may occur [74]. Studies have shown that increased levels of TIMPs may occur in response to increased MMP activity, indicating a compensatory mechanism that may ultimately fail to restore normal turnover of the ECM, impairing its homeostasis [71, 72]. Thus, the balance between TIMPs and MMPs is very important in ocular health by preventing the development and progression of various ocular diseases.

Another aspect to consider regarding susceptibility to glaucoma development is polymorphisms in the *TIMP* and *MMP* genes [48, 75]. Research has demonstrated the existence of specific polymorphisms in the *MMPs* gene, such as in *MMP-9*, being correlated with primary OAG in different population groups. These specific polymorphisms may influence the course of pathogenesis, as genetic predisposition may affect both the expression and activity of TIMPs and MMPs [48, 75]. Studies have demonstrated the importance of TIMPs in the management of inflammatory processes and their impact on MMP and ECM modeling. Due to their ability to decrease elevated MMP levels from inflammatory processes, which can lead to ECM remodeling, TIMPs play a key role in establishing a balance and pathogenesis of chronic glaucoma, helping to significantly improve disease prognosis [76, 77].

Clinically important aspects of glaucoma management with MMPs and TIMPs

MMPs and TIMPs in the pathogenesis of chronic glaucoma

It is well known that both MMPs and TIMPs play a critical role in the management of pathogenesis and progression of chronic glaucoma, and a balance between them is necessary. They mainly influence ECM remodeling and alterations in their expression may lead to negative changes in ocular tissue health.

Clinical studies have helped to elucidate the role of MMPs in glaucoma, and they can be considered biomarkers of the pathogenesis of different forms of glaucoma such as primary OAG and primary ACG, with increased levels of MMPs (especially MMP-2 and MMP-9) observed in the plasma of affected patients [49, 78]. In addition, a direct correlation between MMPs (MMP-2, MMP-9) and ocular proinflammatory processes has been observed, through exacerbation of glaucomatous lesions [79]. It has also been observed that MMP-9 may mediate ECM degradation in glaucoma patients, which is important for the maintenance of normal IOP and overall ocular health, and MMPs may thus contribute to the clinical phenotype of glaucoma [47].

Due to the ability of TIMPs to regulate the activity of MMPs and maintain ECM homeostasis, both TIMPs and MMPs must be in balance to prevent damage to ocular tissues by excessive ECM degradation or excessive ECM accumulation. For example, the study by Fountoulakis *et al.* reported the existence of a compensatory mechanism between TIMPs and MMPs, manifested by increased levels of TIMP-4 in patients with primary OAG due to increased levels of MMPs [70]. However, this compensatory mechanism is not sufficient to prevent glaucoma from worsening, as deregulation of TIMPs and MMPs can lead to remodeling of critical ocular structures such as the *lamina cribrosa* and TM [48, 52]. Studies have shown that the activity of MMPs in conjunctival ocular tissues is increased in patients undergoing glaucoma filtering surgery, suggesting that these enzymes have implications in tissue remodeling and postoperative scarring, with glaucoma patients having altered expression of both MMPs and TIMPs [80]. Furthermore, research such as that of Sahay *et al.* suggests that MMPs could be considered important biomarkers for the diagnosis and prognostic prediction of glaucoma pathogenesis, their expression in tears being correlated with disease severity [79].

Interactions between MMPs and TIMPs

Interactions between TIMPs and MMPs have an important significance in the context of glaucoma pathophysiology, with implications for the TM and IOP regulation, key aspects in glaucoma pathogenesis. Studies show that glaucoma patients have impaired ECM remodeling processes, with MMPs degrading the ECM. In particular, MMP-2 and MMP-9 have been observed in the aqueous humor and conjunctiva of glaucoma patients, contributing to the pathophysiology of glaucoma. Several studies highlight the complex interplay between MMPs and TIMPs, such as the study by Helin-Toiviainen *et al.*, where it is emphasized that if this interaction is out of balance, the pathogenesis of glaucoma may be accentuated [46], while the study by Gupta *et al.* highlights the role that MMPs and TIMPs play in ECM synthesis and in promoting fibroblast migration, with important implications in the processes of scarring and fibrosis in the conjunctiva, and may complicate surgical interventions for glaucoma management [81].

Both TIMPs and MMPs regulate the development of ECM and a balance between them is necessary. TIMPs function to inhibit the proteolytic activity of MMPs, and an increase in MMP activity without a corresponding increase in TIMPs may lead to excessive ECM degradation, whereas increased TIMP levels may inhibit the necessary remodeling of the ECM [59, 82]. In the study by Kara *et al.*, researchers emphasized the vital role of MMP and TIMP expression by TM cells in maintaining leakage resistance, with imbalances between these levels leading to an exacerbation of glaucoma. Another important aspect of the interaction between TIMPs and MMPs is the influence of other factors on the balance between them. For example, cytokines such as TGF-β have been shown to modulate the expression and activity of TIMPs and MMPs. The same group of authors conducted by Kara [83], showed that elevated TGF-β levels correlate with an imbalance between TIMPs and MMPs, manifested by increased MMP expression and reduced TIMP expression, leading to dysregulation of ECM [83]. Subsequently, this ECM dysregulation can lead to pathological changes manifested by impaired aqueous humor outflow and increased IOP, aggravating glaucoma [84].

Future directions in research and therapeutic approaches targeting MMPs and TIMPs

MMPs and their TIMPs are proteins important for both pathological and physiological processes, including

tissue remodeling, inflammation, and tumor progression, making future research directions and therapeutic approaches of these proteins of significant impact in the biomedical field. Recent advances have been made in research on the development of selective inhibitors for MMPs. These inhibitors, particularly those targeting the A-disintegrin and metalloproteinase (*ADAM*) and A-disintegrin and metalloproteinase with thrombospondin motifs (*ADAMTS*) gene families, including monoclonal antibodies and engineered TIMPs, are currently part of clinical trials on the treatment of OA and cancer [85]. The lack of the ability to differentially inhibit MMPs leads to adverse effects due to their involvement in normal physiological processes, and therefore, the specificity of these inhibitors prevents the occurrence of these side effects [86]. For example, N-TIMP2 has been claimed to selectively inhibit MMP-9 and MMP-14, and therefore, improving the therapeutic specificity of these inhibitors is vital for minimizing side effects, and maximizing their therapeutic efficacy [87].

Currently, a novel approach for modulating the expression of MMPs and TIMPs is gene editing technologies, in particular, clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) – a genetic engineering method that allows the modification of certain genes with increased precision in a short time [88]. By using this modern therapeutic technique, it has been possible to effectively manage patients with OA, in whom MMP-13 overexpression plays an important role in the marked and progressive deterioration of bone cartilage [78]. Among the gene-editing genetic engineering modalities, modern medicine also proposes the transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs) system. By precisely targeting and editing genes associated with MMP expression, researchers aim to develop more effective treatments that can halt or reverse the progression of degenerative diseases. Therefore, knowledge and understanding of the molecular mechanisms underlying MMP regulation and their interactions with other signaling pathways, such as those involving inflammatory cytokines, is required for the use of these technological editing approaches [89].

MMPs are involved in immune cell migration and function [90] and therefore their role in immune cell biology and influence on tumor microenvironments highlight their potential use as therapeutic targets. For example, Kwon demonstrated that modulation of the immune micro-environment by MMP inhibition leads to increased efficacy of immunotherapy in breast cancer [91]. Therefore, Kwon's study suggests that combining MMP inhibitors with existing immunotherapeutic strategies produces synergistic effects leading to improved patient outcomes.

Another innovative therapeutic strategy is the use of nanotechnology to deliver MMP inhibitors. For example, Ji *et al.* [92] have developed drug-loaded supramolecular nanofibers to inhibit MMP activity. The authors' results showed that drug-carrying nanofibers have therapeutic potential in reducing liver metastasis [92]. Therefore, this type of approach improves targeted drug delivery therapy while minimizing systemic toxicity, which continues to be still a challenge in cancer treatment.

In addition to their role in cancer, MMPs also play a role in neurodegenerative diseases (*e.g.*, AD). Zipfel *et al.* have shown that MMPs can modulate neuroinflammation and neuroplasticity, making them attractive targets for therapeutic interventions in neurological disorders [93].

At the moment, as a future research direction but also a challenge for researchers, maybe the development of inhibitors that can efficiently cross the blood–brain barrier while retaining their specificity so that disruption of normal brain function is avoided.

Conclusions

Chronic glaucoma is a complex disease characterized by progressive optic nerve damage and irreversible vision loss. The mechanisms involved in the pathophysiology of glaucoma are elevated IOP levels, neuroinflammation, oxidative stress, and vascular factors, respectively. To develop effective treatment strategies leading to improved patient outcomes, a full understanding of these mechanisms is necessary. Risk factors that contribute to the onset and progression of glaucoma include age, family history, systemic health status, lifestyle, and specific ocular characteristics. These risk factors are major contributors to the detection, diagnosis, and effective management of glaucoma to mitigate its impact on vision.

MMPs are enzymes that mediate ECM remodeling and contribute to the regulation of inflammation, wound healing, and cancer. MMP-2 and MMP-9 are the most important enzymes that contribute to the development of chronic glaucoma through physiological mechanisms, being involved in ECM degradation, inflammation, and genetic predisposition. These two enzymes can be used both as therapeutic strategies against glaucomatous lesions and as biomarkers to assess disease progression.

TIMPs influence normal physiological processes and pathological disease conditions, as these inhibitors regulate MMP activity in the eye through complex mechanisms. With the understanding of these mechanisms, new therapeutic strategies can be addressed to restore the balance between TIMPs and MMPs, thus leading to improved ocular health. Therefore, future research strategies should focus both on the establishment of these complex mechanisms that determine precisely how TIMPs exert their effects on MMPs and their use in different diseases as therapeutic targets. It is important to discover in as much detail as possible the mechanisms underlying the pathophysiology of glaucoma to develop the most effective therapies that are able to restore ECM homeostasis disturbed by the imbalance between MMPs and TIMPs and to help prevent an increase in IOP.

Conflict of interests

The authors declare no conflict of interests.

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