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Elastin turnover in ocular diseases: A special focus on age-related macular degeneration

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Abstract

The extracellular matrix (ECM) and its turnover play a crucial role in the pathogenesis of several inflammatory diseases, including age-related macular degeneration (AMD). Elastin, a critical protein component of the ECM, not only provides structural and mechanical support to tissues, but also mediates several intracellular and extracellular molecular signaling pathways. Abnormal turnover of elastin has pathological implications. In the eye elastin is a major structural component of Bruch's membrane (BrM), a critical ECM structure separating the retinal pigment epithelium (RPE) from the choriocapillaris. Reduced integrity of macular BrM elastin, increased serum levels of elastin-derived peptides (EDPs), and elevated elastin antibodies have been reported in AMD. Existing reports suggest that elastases, the elastin-degrading enzymes secreted by RPE, infiltrating macrophages or neutrophils could be involved in BrM elastin degradation, thus contributing to AMD pathogenesis. EDPs derived from elastin degradation can increase inflammatory and angiogenic responses in tissues, and the elastin antibodies are shown to play roles in immune cell activity and complement activation. This review summarizes our current understanding on the elastases/elastin fragments-mediated mechanisms of AMD pathogenesis.

Keywords

Age-related macular degeneration; Extracellular matrix; Elastin; Elastase; Elastolytic enzymes; Elastin-derived peptides; Inflammation; Immune cell activity; Complement activation

1. Introduction

Age-related macular degeneration (AMD) is a major cause of irreversible vision loss among the elderly, currently affecting 170 million people worldwide (Pennington and DeAngelis, 2016). The United Nations 2019 report documented 703 million persons aged 65 years or older in the world, a number that is projected to double by 2050 (https://www.un.org/en/development/desa/population/publications/pdf/ageing/WorldPopulationAgeing2019-Highlights.pdf). Based on the current percentages of

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25% early or intermediate AMD and 2.4% late AMD prevalence in the European Population 60 years or older (Li et al., 2020), or 0.37% late stage AMD prevalence in the 45-85-years-old global population (Schultz et al., 2021), the number of AMD patients is expected to soar. AMD is characterized by the central vision loss caused by the degeneration of rod and cone photoreceptors in the macula (Curcio et al., 1996), the area of the retina with the highest cone density, enabling central vision. Main risk factors of AMD include aging (Hussain et al., 2010), cigarette smoking (Seddon et al., 1996; Thornton et al., 2005; Velilla et al., 2013), oxidative stress, cardiovascular diseases, and genetic variants (Lim et al., 2012), which include mutations of genes regulating the inflammatory (Klein et al., 2005; Rohrer et al., 2019) and the extracellular matrix (ECM) turnover pathways (Kondo et al., 2008; Yamashiro et al., 2011).

Macular photoreceptor degeneration in AMD is mainly driven by the reduced or failing support from the outer retinal components, such as the retinal pigment epithelium (RPE)/Bruch's membrane (BrM)/choriocapillaris (CC) complex. Based on the progression of the disease AMD is classified into two forms: dry and wet. In dry AMD or geographic atrophy (GA), RPE dysfunction appears first, followed debilitation of photoreceptors and choriocapillaris. In wet AMD, also called neovascular (n)AMD or exudative AMD, loss or dysfunction of the choroidal vasculature occurs first (Bhutto and Lutty, 2012), resulting in subsequent neovascularization, RPE dysfunction, and photoreceptor cell loss. The most prevalent form of AMD is dry AMD with severe cases of GA in the central retina, (Fleckenstein et al., 2018). Although wet AMD represents only 10–15% of total AMD cases, it accounts for ~90% of the blindness or severe vision loss associated with AMD (Ambati and Fowler, 2012; Ferris et al., 1984).

The underlying molecular mechanisms of AMD are not yet fully understood; thus, limited treatment strategies are available. Intravitreal injections of anti-VEGF are the only current FDA-approved treatment available for nAMD (Bloch et al., 2012). While anti-VEGF treatments show significant short-term benefits on best-corrected visual acuity and retinal structure in nAMD, continuous anti-VEGF exposure can cause drug intolerance, increased fibrosis, occasional development of GA, and hence fails to have long-term benefits in patients (Binder, 2012; Kaynak et al., 2018). Additional treatment paradigms include, but are not limited to, complement inhibitors that have been studied in intermediate AMD and late-stage GA (Wu and Sun, 2019) or an autophagy inducer such as rapamycin (sirolimus) tested to improve late-stage, persistent nAMD (Ambati and Fowler, 2012; Dejneka et al., 2004; Ishikawa et al., 2015) or GA (Gensler et al., 2018). However, other than anti-VEGF therapeutics, no other pathways have been proven as an effective strategy to reduce the progression of AMD. Therefore, a better understanding of AMD-associated pathways is crucial to developing an effective treatment strategy.

Aging and its associated accumulation of damage at the ECM are major risk factors for AMD. During aging many changes occur at the ECM including the accumulation of protein aggregates, ECM component crosslinking, glycation or fragmentation, with every cell type and tissue type producing its own unique ECM (Ewald, 2020). Studies have shown that damage of ECM or its abnormal turnover, especially at the RPE/BrM/CC complex has significant role in the pathogenesis of AMD (Nita et al., 2014). BrM is a complex

five-layered elastin and collagen rich ECM structure located between RPE and CC, and is composed of the RPE basal lamina (RPE-BL), the inner collagenous later (ICL), the elastin layer (EL), the outer collagenous later (OCL) and the choriocapillaris basal lamina (CC-BL) (Curcio and Johnson, 2013). Degradation of EL at the macular BrM has been reported in AMD patients, and this EL loss has been postulated to enable choroidal blood vessels to break through BrM and cause CNV (Chong et al., 2005). Overall, EL thickness is less in the macula than in the periphery; and even less thick in eyes with early AMD and active CNV. Thus, this reduction in BrM EL integrity, which is important for BrM's biomechanical properties and for maintaining a physical barrier to prevent the invasion of blood vessels, might provide some rationale why CNV occurs in the macula (Chong et al., 2005). In connection with this observation, serum elastin-derived peptides (EDPs), the byproducts of elastin degradation are significantly higher in moderate-severe AMD patients, especially AMD patients with retinal vascular abnormalities (Sivaprasad et al., 2005). Correspondingly, elevated levels of α-elastin antibodies (Morohoshi et al., 2012) and elastin gene polymorphisms are also reported in nAMD patients (Kondo et al., 2008; Yamashiro et al., 2011). Together these lines of evidence suggest that elastin metabolism plays a critical role in the pathogenesis of AMD and retinal angiogenesis. This review summarizes our current understanding of the role of elastin turnover in dry and wet forms of AMD.

2. Elastic fiber biogenesis, turnover and elastase inhibitors

Elastic fibers are made up of two distinct ECM components: elastin and microfibrils. The fibrous protein elastin makes up 90% of elastic fiber, forming its internal core, and the microfibrils provide a scaffold and organize the core elastin to form the typical elastic fiber. Elastin is encoded by a single mRNA transcript of 3–5 kb size (Davis and Mecham, 1998). The translated tropoelastin monomers are chaperoned by elastin binding protein (EBP; 67 kDa), which prevents intracellular self-segregation and premature degradation of elastin. The intracellularly formed tropoelastin monomers are then transferred to the extracellular space, where they will be polymerized to insoluble mature elastic fibers with the help of cross-linking enzymes lysyl oxidases (LOX) and LOX-like proteins (LOXL) 1–4(Mithieux and Weiss, 2005).

Microfibrils are mainly made up of fibrillin 1, fibrillin 2, and microfibril-associated glycoprotein-1 (MAGP-1) (Brown-Augsburger et al., 1996; Mithieux and Weiss, 2005; Sakai et al., 1986; Zhang et al., 1994). Another major group of ECM glycoproteins involved in elastin fiber assembly are fibulins-4 and −5. Fibulin-4 contributes to elastin cross-linking by activating and tethering LOX to tropoelastin (Horiguchi et al., 2009), and fibulin-4 deficient mice have disrupted elastin and collagen fibers and die shortly after birth (Noda et al., 2020). Interactions between fibulin-5 and tropoelastin as well as LOX, LOXL 1, 2 and 4 have been also reported. Fibulins 4, 5 can also bind to fibrillin 1 (Godwin et al., 2019). Together these non-redundant and essential interactions between fibulins, LOX, tropoelastin and fibrillins facilitate the formation of highly crosslinked insoluble elastic fibers (Fig. 1). Extensive cross-linking makes elastin one of the extremely durable proteins in the body with a half-life of ~70 years (Petersen et al., 2002) and minimal turnover rate in the body, with

two exceptions, pathological conditions, or aging. Factors that affect elastin turnover during disease and aging are still emerging.

Major factors that affect elastin turnover at the post-translational level are the elastases and their inhibitors. Elastases are defined as the elastin solubilizing enzymes as they can degrade insoluble mature elastic fibers and highly crosslinked elastin polymers into soluble elastin fragments (Bieth, 1986) (Fig. 1). Based on the differences in catalytic sites and enzyme activity elastases can be of serine, cysteine, or metalloproteinase category (Table 2). Major elastases, such as pancreatic and neutrophil elastases fall under the category of serine proteinases, whose catalytic activity depends on the serine group present on their catalytic sites and can be inhibited by alpha 1 proteinase inhibitors. Elastases produced by macrophages, which are the phagocytes derived from circulating monocytes, fall under the category of matrix metalloproteinase 12 (MMP12). Unlike serine protease, MMP12 is resistant to the inhibition by alpha-1 proteinase inhibitors (Banda and Werb, 1981; Werb et al., 1982), but their activity can be blocked by tissue inhibitors of metalloproteinases (TIMPs 1–4) and also by chelating agents such as EDTA, as they require metallic presence for their catalytic activity (Brew and Nagase, 2010).

Studies indicate that inflammation is a major trigger for increased elastase activity. TNF-α increases elastase activity in smooth muscle cells (Kothapalli and Ramamurthi, 2010), and IL-1β exposure increases elastase activity in RPE (Elner, 2002) and fibroblast cells (Croute et al., 1991). In addition, increased elastase activity can further exacerbate inflammation. It has been shown that neutrophil elastase increases insulin resistance and inflammation in mouse hepatocytes and adipocytes, and in support of this data, under conditions of high-fat diet and obesity, neutrophil elastase deficient mice exhibited increased insulin sensitivity and reduced inflammatory response (Talukdar et al., 2012). Thus, inflammation and elastase activities are interconnected, and can function as major triggers of abnormal elastin turnover in aging and diseases.

2.1. Neutrophil elastase inhibitors

Human neutrophil elastase is a basic, 218 amino acid single polypeptide glycoprotein with a molecular weight of 29.5 kDa. Its primary structure shows considerable homology with proteinase 3 (54%) and cathepsin G (37%). There are some endogenous protein inhibitors of elastase that include the two serpins, the α-1-proteinase inhibitor (α-1-PI) and monocyte/neutrophil elastase inhibitor (Serpin B1), and the chelonianin family of canonical inhibitors, secretory leukocyte proteinase inhibitor (SLPI) and elafin (Groutas et al., 2011). Elastase inhibitors have been the focus for drug development due to the involvement of elastases in pathologies of several prevalent diseases including chronic obstructive pulmonary disease (COPD) and fibrosis. However, it has been a challenge to specifically target the activity of elastase, due to its similarity and its overlapping functions with other serine proteases. A review article provides “an overview of major developments in COPD research with emphasis on low molecular weight neutrophil elastase inhibitors”, and the readers are referred to this excellent work (Groutas et al., 2011). In short, the design has focused on the development of so-called transition-state inhibitors, that bind to the enzyme’s active site serine and thereby prevent activation, or on agents that acylate the active site

serine and have shown efficacy in the rat acute lung injury model (Groutas et al., 2011). Recently several anti-elastase polypeptides of bacterial, fungal, plant, and animal origin have been reported. These polypeptides are cyclic depsi-peptides containing modified and unusual amino acid residues, which developed as inhibitors of digestive enzymes and chemical defense against predators. These polypeptides have only been tested in vitro and are awaiting in vivo functional assays (Ahmad et al., 2020). Currently, the three FDA-approved neutrophil elastase inhibitors include Prolastin (Bayer), Aralast (Baxter), and Zemira (Aventis Behring), which are human plasma-derived alpha 1-antitrypsin (A1AT) therapies. A1AT is a 52 kDa serpin and was first described as a trypsin inhibitor; however, it acts as a more general potent protease inhibitor that can also limit elastase activity (Stockley, 2015).

3. Elastin in Bruch's membrane (BrM) and other ocular tissues

In the eye, elastin is widely distributed and has been detected in the cornea, conjunctiva, muscle tendons, sclera, choroid, BrM, meninges (Gelman et al., 2010), lamina cribrosa (Hernandez et al., 2000) and retina (Chen and Weiland, 2014). In the anterior segment of the eye, elastin is critical for maintaining the shape of the cornea (Feneck et al., 2018; White et al., 2017a) and for controlling the outflow resistance in the trabecular meshwork (Umihira et al., 1994). Posteriorly, elastic fibers of the sclera protect the globe from mechanical stresses like elevated intraocular pressure (Gelman et al., 2010) and those present at the BrM appear to work as a structural barrier preventing choroidal vessels from growing into the outer retina (Chong et al., 2005). In human BrM, elastic fibers are stacked together with an interfibrillar space of ~1 μm to form a perforated elastin sheet or elastin layer (EL). This elastin sheet spans from the optic nerve to the pars plana of the ciliary body. In addition to elastin, the BrM EL also contains other ECM proteins such as collagen type VI and fibronectin. Human BrM thickness is reported as 2–4 μm, the EL making up the central 0.8 μm; similarly, in murine models, a total BrM thickness of 0.22–0.64 μm has been reported (Annamalai et al., 2020; Volland et al., 2015), with apparently a larger component of the overall thickness being contributed by the EL (0.13–0.17 μm) (Volland et al., 2015).

Human BrM forms by 6–7 weeks of gestation, with the BrM EL forming and becoming histologically visible by 11–12 weeks. Embryologically, RPE-BL and ICL are derived from ectoderm, OCL and CC-BL from mesoderm, with the EL being deposited by invading fibroblasts and endothelial cells of the CC (Curcio and Johnson, 2013). Consistently, tropoelastin mRNA levels are significantly higher in the embryonic and early postnatal days in murine eyes involving two phases of EL development, BrM elastin layer formation followed by maturation (Mori et al., 2019).

RPE has a crucial role in synthesizing several ECM proteins that make up BrM, including collagen I, III, IV, V, fibronectin, and laminin (Newsome et al., 1988). Although the RPE's role in BrM elastin synthesis and degradation is not clearly stated, some studies performed in cultured RPE cells give us some clues. Cultured human RPE cells (ARPE 19 cells) express fibrillin 1 and LOX, suggesting that they can facilitate elastic fiber assembly (Wachi et al., 2005; Yeo et al., 2012), although the elastin gene expression is minimal (Sharma et al., 2005). Also, tropoelastin mRNA and protein expression has been reported in heat

exposed ARPE19 cells, suggest their contribution in elastogenesis under stress (Sekiyama et al., 2012), and single cell RNA-seq profiling data demonstrated elastin expressing in freshly isolated primary human RPE cells (Dwight Stambolian, personal communication, 3-11-2022). Additionally, a single-cell RNA sequencing analysis performed on infant and aged RPE/choroid samples has reported a ~10-fold higher elastin (ELN, chromosome 7) gene expression in infant pericytes compared to the aged samples, supportive of the pericyte's contribution to elastin production and reemphasizing the early occurrence of elastogenesis in the eye during development (Voigt et al., 2020).

Elastin makes up ~4% of the dry retinal tissue weight and has been detected at the basement membrane of retinal arterial walls (Chen and Weiland, 2014; Chong et al., 2005; Sagaties and Raviola, 1989). It has been suggested that smooth muscle cells and endothelial cells of retinal vessel walls might be involved in retinal elastin synthesis as they stain positive for elastin (Chen and Weiland, 2014). Another major retinal cell type that expresses elastin are the astrocytes present at the lamina cribrosa, which express elastin during development as well as in glaucoma (Hernandez et al., 2000; Pena et al., 2001).

4. Elastin turnover in ocular diseases

Abnormal elastin turnover/elastic fiber degradation/elastase activity has been associated with several ocular diseases (Table 1). Elastic fibers distributed throughout the sclera and the cornea play critical role in maintaining the structural integrity of the eye globe. Scleral elastin is most dense in the peripapillary region or near the optic nerve head and is predicted to have role in maintaining the intraocular pressure (Gelman et al., 2010). In primary open-angle glaucoma (POAG) (Hernandez, 1992), elastin loss and fragmentation are reported at the lamina cribrosa of the optic nerve head, a tissue required to maintain the ocular pressure gradient. Additionally, reduced LOX1, elastin protein expression and structural alterations of the elastic fiber network are reported in the lamina cribrosa of pseudoexfoliation glaucomatous eyes, a type of POAG which is caused by the abnormal deposition of proteins in the drainage system or other parts of the eye (Schlötzer-Schrehardt et al., 2012).

In POAG abnormal elastin turnover has also been reported at the trabecular meshwork (TM) and Schlemm's canal endothelial cells, tissues which are critical for maintaining the aqueous outflow resistance. In particular, the elastin fiber sheath in the TM has been proposed to play a significant role in managing outflow resistance (Segawa, 1995), as thickening of the sheath has been observed in the aged TM. Age-related TM elastic fiber thickening may increase the risk of POAG in aging population (Tektas and Lütjen-Drecoll, 2009). Moreover, increased elastin expression has been reported in Schlemm's canal endothelial cells of POAG patients compared to the age matched healthy eyes (Umihira, 1993; Umihira et al., 1994). Collectively, these data suggest that abnormal elastin turnover and its deposition at the TM/Schlemm's canal complex could contribute in POAG pathogenesis by increasing the aqueous outflow resistance.

Upregulated elastolytic enzymes (MMP7 and MMP9), elastic fiber damage and reduced elastin levels are evident in floppy eyelid syndrome, an eye disease characterized by

the diminished mechanical properties of the upper eyelid (Netland et al., 1994; Schlötzer-Schrehardt et al., 2005).

Corneal thinning (White et al., 2017a), ectopia lentis/dislocation of the lens (Zadeh et al., 2011), retinal detachment (Dietz, 1993), and impairment of retinal and choroidal vasculature (Di Marino et al., 2020) are reported in patients with Marfan syndrome, a hereditary connective tissue disorder caused by the mutation of fibrillin-1, a key microfibril protein essential for elastic fiber assembly.

Studies indicate that abnormal elastin turnover is a key player in keratoconus pathogenesis, a corneal disorder that is associated with progressive corneal thinning, ultimately leading to astigmatism and vision loss. Abnormal microfibril bundle distribution and the absence of the elastic fibril system reported in the posterior stroma of keratoconus corneas (White et al., 2017b) might be associated with the decreased distribution of LOX protein, and LOX gene polymorphisms (Dudakova and Jirsova, 2013; Dudakova et al., 2012; Hasanian-Langroudi et al., 2015). Reduced corneal transcript levels of LOX, together with reduced LOX activity in keratoconus patient tears have been associated with disease severity, as were reduced collagen and increased MMP9 transcript levels (Shetty et al., 2015).

Age-related visual impairment, increased BrM EL calcification, foveal CNV, macular atrophy and outer retinal atrophy and RPE atrophy (Gliem et al., 2016) are reported in pseudoxanthoma elasticum (PXE) pathogenesis (Jensen, 1977; Risseeuw et al., 2019, 2020a), a rare multi-system genetic disorder that primarily accompanies with increased calcification and fragmentation of elastic fibers (Roach and Islam, 2015). Cardiovascular, dermatological, and ophthalmological symptoms are common in these patients. The BrM breaks or angioid streaks due to elastin calcification (Charbel Issa et al., 2009) were co-localized with the CNV lesions and macular atrophy in PXE (Risseeuw et al., 2020b), indicating that BrM EL integrity is important for RPE survival and for preventing pathological CNV.

Sorsby's Fundus Dystrophy (SFD), an ocular disease caused by mutations in the TIMP3 gene, shares many clinical features with AMD. Accumulation of mutated TIMP3 at BrM, and a thickening of BrM and BrM elastin layer abnormalities/abnormal arrangement of elastic fiber components, are reported in SFD pathogenesis along with CNV (Anand-Apte et al., 2019; Chong et al., 2000; Gourier and Chong, 2015). The elastin layer of BrM is irregular, thickened and broken in SFD compared to healthy subjects (Chong et al., 2000), indicating that mutated TIMP3 in SFD is failing to protect the EL or it has abnormal functions. BrM EL damage caused by the TIMP3 mutation may contribute to CNV invasion in SFD (Gourier and Chong, 2015).

Elastase activity and elastin turnover are also critical in diabetic retinopathy. Decreased LOX activity and increased MMP levels (MMP2, MMP9) are reported in the vitreous of proliferative diabetic retinopathy and rhegmatogenous retinal detachment, diseases for which inadequate collagen cross-linking and ECM alternations have been reported (Coral et al., 2008). Diabetes increases neutrophil elastase levels in both the retina and serum. Increased anti-elastin IgA antibodies were found in pediatric diabetic retinopathy patients

(Nicoloff et al., 2000). Based on these observations, elastase inhibitors such as A1AT have been suggested as a possible therapeutic strategy for diabetic retinopathy. Interestingly, intraperitoneal administration of A1AT improved retinal thickness and retinal ganglion cell (RGC) loss in an animal model of diabetic retinopathy, likely via attenuating the level of pro-inflammatory cytokines such as TNF-α produced by macrophages (Ortiz et al., 2014, 2018; Potilinski et al., 2020). On the other hand, A1AT deficiency is associated with optic neuropathy (Younger et al., 2006) as well as ocular inflammatory conditions such as conjunctivitis and uveitis (Leonardi et al., 2010). Interestingly, deletion of IL-17 significantly reduced diabetes-induced increase of neutrophil elastase in retina and serum (Liu et al., 2019), indicative of cytokine modulation of elastin turnover in the diabetic retina.

A plethora of studies mentioned above emphasize a strong correlation of abnormal elastin turnover with multiple ocular diseases. One critical question arising here is that whether the elastin loss/disruption/abnormalities are part of the disease pathogenesis or a primary cause. Current evidence suggests a significant role of elastin turnover in the etiology of ocular diseases, as well as that altered biomechanics of elastic fibers and the inflammatory or the proangiogenic role of EDPs (Figs. 1 and 3) generated by the perturbed elastin turnover/ increased elastase activity, could contribute to the pathology of ocular diseases.

5. Elastin turnover and elastase activity in AMD

Thickening of BrM, and the relative decrease of elastin and increased calcification of EL are characteristics of aging BrM along with lipid buildup (Curcio, 2018; Curcio et al., 2011), concomitant with the development of basal laminar deposits (BLamD; localized between the RPE basement membrane and its plasma membrane) (Sarks, 1976) and basal linear deposits (BLinD; localized between the RPE basement membrane and the inner collagenous zone) (Sarks et al., 1980). Reduced elasticity (Booij et al., 2010) and macromolecular diffusion characteristics of the aging BrM caused by these structural alterations (Hussain et al., 2010) can contribute to RPE dysfunction and make the retina susceptible to CNV (Chong et al., 2005) (Fig. 2). A pathological increase of MMPs with elastase activity can exacerbate the age-related elastic fiber degradation and calcification (Basalyga et al., 2004) leading to increased brittleness of BrM and AMD pathology. Evidence indicates that abnormal elastin turnover has a significant role in the pathogenesis of wet AMD and pathological angiogenesis, but its role in dry AMD cannot be excluded. In disease, RPE cells (Elner et al., 2003), infiltrating immune cells such as neutrophils and macrophages (Beguier et al., 2020; Ghosh et al., 2019; Liu et al., 2019; Nakajima et al., 1992), as well as microglia (Ma et al., 2012; Nakajima et al., 1992) can upregulate their elastase secretion in the outer retina. This can exacerbate the age-related elastin degradation, and may contribute to both wet and dry AMD pathology (Fig. 3).

5.1. Elastin turnover in dry AMD

In the normal healthy retina, the mean macular EL thickness at the BrM is approximately 1/3 of the thickness of the mean extramacular EL thickness (Chong et al., 2005). The integrity of the EL, which is defined as the total length of visually detectable elastin divided by the overall length of visible BrM, is almost 100% in the extramacular region

but drops to ~40% in the macula itself. In early AMD, the integrity of BrM EL is significantly reduced compared to age-matched healthy controls, whereas in late-stage AMD patients, including donors with choroidal neovascular membranes, both macular BrM EL integrity and thickness are significantly reduced compared to the controls, with a strong relationship between these two parameters in all regions measured. Surprisingly, this pathology-related decrease in macular elastin at the BrM was not evident in AMD patients with GA, a phenomenon that is currently not understood (Chong et al., 2005). Nevertheless, significantly reduced elastin loss and consequent vessel hardening/inelasticity are reported in the retinal vessels of dry AMD (GA) patients compared to the healthy control subjects (Chen and Weiland, 2014). Authors interpret that the retinal vessel damage caused by abnormal elastin turnover could contribute to fluid accumulation and impaired waste removal in the retina, leading to drusen deposition and GA progression.

Together, these findings suggest that loss of mechanical integrity of the EL, as well as the inflammation triggered by production of elastin peptides, might play a role in dry AMD progression. A thinner and more porous EL could lead to impaired RPE attachment to its basement membrane, promoting detachment of RPE cells, accumulation of basal laminar deposits as well as drusen formation (Chong et al., 2005). This hypothesis is supported by the use of elastin-based recombinant polymers that provide a good substrate for RPE cells (Shadforth et al., 2015; Sreekumar et al., 2018; Srivastava et al., 2011), indicating that degradation of the EL in BrM could lead to RPE dysfunction/detachment and could contribute to dry AMD pathogenesis. Evidence from animal studies also support the contribution of abnormal elastin turnover in dry AMD progression. A cigarette smoke induced dry AMD mouse model exhibited thickened BrM, especially outer collagenous layer thickening along with BrM elastin loss (Annamalai et al., 2020). EDPs are reported to increase collagen depositions in the retina (Skeie et al., 2012), suggesting that EL degradation and consequent production of EDPs could be a cause of increased collagen deposition leading to BrM thickening reported in AMD retinas. Also, mutations of fibulin-3 and −5, proteins involved in elastic fiber formation (Nakamura et al., 2002), have been associated with dry AMD characteristics, such as RPE detachment and sub-RPE drusen formation (Stone et al., 2004). Honeycomb retinal dystrophy, a retinal disease exhibiting many of the characteristics of dry AMD such as BrM thickening, sub-RPE depositions, and choroidal/choriocapillaris atrophy, along with photoreceptor cell disruption in the subfoveal space, is caused by a single missense mutation in fibulin-3 (Fu et al., 2007; Gerth et al., 2009). Fibulin-3 interacts with several ECM proteins including tropoelastin and collagen, and it stimulates the expression of TIMP 1 and 3, and inhibits the activities of MMP 2, 3 and 9 as well as angiogenesis (Zhang and Marmorstein, 2010). Whether fibulin-3 mutations are merely associated with drusen formation or whether its elastase inhibitory effect also contributes to dry AMD pathogenesis is not yet clear.

5.2. Role of elastin turnover and elastases in RPE cells

At the posterior eye, the RPE could play a critical role in elastin remodeling. The RPE cells secrete many of the ECM proteins needed for the generation of RPE basal lamina and regulate its remodeling with protease enzymes and their inhibitors (TIMPs 1, 2) (Alexander et al., 1990). RPE cells secrete low levels of several enzymes that have

collagenase (MMP1), gelatinase (MMP2 and MMP9), and elastase activities (Guo et al., 1999; Hollborn et al., 2007). Cultured RPE cells secrete metalloproteinases and tissue inhibitors of metalloproteinases (TIMPs) into the media (Alexander et al., 1990), and in polarized monolayers, specifically towards the apical side (Greene et al., 2017; Kay et al., 2013).

Studies have shown that oxidative stress and cytokine exposure can modulate the elastase secretion from the RPE. Oxidative stress upregulates MMPs 1 and 3 levels in RPE cells (Alge-Priglinger et al., 2009) without altering the expression of TIMP-1. Similarly, TNF-α, IL-1β, and TGF-β2 exposures also increase the expression of MMP1, MMP2, and MMP3 and increase elastase activity (Elner et al., 2003) in cultured human RPE cells (Eichler et al., 2002). Elastases secreted by the RPE are not only involved in the elastic fiber breakdown, but can also contribute to retinal angiogenesis. Notably, increased MMP9 activity in cultured RPE cells induced by complement activation (H_2O_2 plus normal human serum) is associated with increased mobilization of VEGF and decreased mobilization of PEDF from the RPE ECM (Bandyopadhyay and Rohrer, 2012).

5.3. Role of elastin turnover in angiogenesis and wet AMD

Interestingly, Blumenkranz and colleagues have observed a correlation between wet AMD and an increased susceptibility of elastic fibers in general to photic or other degenerative stimuli (Blumenkranz et al., 1986). Several studies later supported this observation by reporting significant associations between elastin gene (ELN) polymorphisms and AMD pathogenesis, particularly in wet AMD (Kondo et al., 2008; Tanaka et al., 2011; Yamashiro et al., 2011; Yanagisawa et al., 2015). Additionally, the association of a polymorphism in HTRA1, an enzyme with elastase-like activity with all forms of AMD (Beguier et al., 2020; Cameron et al., 2007; Grob et al., 2012; Martínez-Velasco et al., 2020; Mohamad et al., 2019; Sundaresan et al., 2012; Tang et al., 2009; Tuo et al., 2008), and an HTRA1 promoter polymorphism association with wet AMD (Dewan et al., 2006), further supports the importance of elastin metabolism in wet AMD. It is interesting to investigate why elastin metabolism abnormalities are more prominent in wet AMD cases compared to dry AMD.

RPE-specific overexpression of HTRA1 in mouse RPE provides some mechanistic clues. Overexpression of human HTRA1 was associated with degradation of BrM elastin (Jones et al., 2011; Vierkotten et al., 2011), in combination with smoke exposure resulted in CNV (Nakayama et al., 2014), triggered PCV, a variant of wet AMD (Jones et al., 2011), and promoted inflammation and macrophage infiltration essential for ocular VEGF expression (Lu et al., 2019). In this mouse model, the authors observed the development of PCV in aged mice, and documented that elastase activity and proteolytic degradation triggered the initiation of CNV, whereas PCV progression was driven by the elevated immune response (Kumar et al., 2017). This data suggests multiple concepts about why abnormal elastin metabolism might be more important in wet AMD. First, weakening of the BrM EL by increased elastase activity together with increased elastin degradation evident in normal aging, can weaken the macular BrM, enabling CNV. Or, second, elastases and EDPs exhibit a varying range of pro-inflammatory and pro-angiogenic roles (Antonicelli et al., 2007) that could trigger CNV or PCV.

HTRA1 is a multifunctional serine protease produced by the RPE and secreted basally towards the choroid (Jones et al., 2011). In addition to its ability to degrade the elastic lamina of BrM and choroidal blood vessels (Jones et al., 2011), it can also cleave EFEMP1, an extracellular matrix protein associated with Doyne honeycomb retinal dystrophy, and thrombospondin 1 (TSP1), an inhibitor of angiogenesis (Lin et al., 2018). Surprisingly, HTRA1 protein levels have been found to increase with age in the RPE-BrM interface (Williams et al., 2021), but only in eyes from donors without the SNP in Chr10 (nonrisk). In donors with homozygous risk at the 10q26 locus, this age-related increase fails to occur, suggesting that loss of function is associated with AMD risk. These results are contrary to those obtained in mouse models, in which expression of human HTRA1 in RPE is associated with pathology (Kumar et al., 2014; Nakayama et al., 2014; Vierkotten et al., 2011). Likewise, the stated goals of the current phase II clinical trial by Genentech, which aims to inhibit HTRA1 in GA using blocking antibodies (NCT03972709), imply that ain of function is associated with AMD risk.

The study performed by Chong and colleagues (Chong et al., 2005) shows that areas that lack structural integrity of BrM EL corresponded to the sites of macular lesions in AMD patients. Hence a disruption at the level of BrM, may cause a breach of the barrier, triggering or enabling choroidal endothelial cells to migrate and form CNV. Experimental laser photocoagulation models confirm this hypothesis that disruption of BrM can induce CNV. The data by Schwesinger and colleagues confirm the notion that angiogenesis alone is not sufficient to induce CNV; their study showed that VEGF-induced angiogenesis leads to CNV only in the presence of a disrupted BrM (Schwesinger et al., 2001). Additionally, mice lacking LOXL-1 an enzyme essential for elastin polymerization have fragmented BrM EL and are more susceptible to CNV lesions following laser induction compared to the wild type with an intact EL (Yu et al., 2008).

Consistent with significant BrM elastin loss in the CNV eye, significantly higher serum levels of EDPs (Sivaprasad et al., 2005) and elastin antibodies (Morohoshi et al., 2012) are reported in wet AMD patients compared to the non-disease controls and dry AMD subjects. It has been shown that EDPs generated from vascular elastin degradation by proteases, attract T cells and macrophages that can contribute to vascular smooth muscle cell differentiation, proliferation and migration, overall contributing to vascular occlusive diseases (Brooke et al., 2003; Karnik et al., 2003). Similarly, EDPs generated from the degradation of BrM EL or retinal vessels could increase inflammation and angiogenesis in the AMD eye. Direct effects of EDPs have also been shown in endothelial cells, as EDPs increase cell migration, tubulogenesis, and proangiogenic MT1-MMP expression in choroidal endothelial cells (Gunda et al., 2013; Skeie and Mullins, 2008). These EDP-mediated angiogenic responses in choroidal endothelial cells could be prevented by the exposure of collagen fragments (Gunda et al., 2013), suggesting the opposite roles of elastin and collagen fragments in retinal angiogenesis. Moreover, it has been shown that circulating EDPs can bind to the beta-galactosidase (GLB-1) receptor present on choroidal endothelial cells and contribute to increased deposition of collagen IV in the RPE/choroid (Skeie et al., 2012), which ultimately can lead to BrM thickening.

Additionally, mutations of genes essential for elastic fiber formation are also associated with CNV (Lamande and Bateman, 2020). Mutations in EGF-containing fibrillin-like ECM protein 1 (EFEMP1) lead to Doyne Honeycomb Retinal Dystrophy, a disease in which drusen formation can lead to CNV (Terai et al., 2011); mutations in FBLN5, which leads to Cutis laxa, a disease characterized by loose skin, is associated with cuticular drusen, retinal detachment and CNV (Lotery et al., 2006). The deficiency of TIMP-3, an inhibitor of several MMPs with elastase-like activity has been reported in wet AMD patients (Krogh Nielsen et al., 2019) and RPE-specific overexpression of TIMP3 interestingly has been shown to ameliorate CNV in experimental models of AMD (Takahashi et al., 2000). Finally, anti HTRA1 antibody has been shown to reduce the lesion size in the photocoagulation murine models of CNV (Lu et al., 2019). Taken together, modulating elastin turnover via the elastase inhibitors appears to be a potential target in wet AMD.

A critical question already mentioned above is whether abnormal elastin turnover associated with AMD is an age-related phenomenon or a causative factor. Although aging can contribute to elastin turnover abnormalities, BrM elastin layer degradation or elastin turnover abnormalities reported in AMD (summarized in this review) are not just age-related occurrences. Data indicate more specific and prominent BrM changes such as reduced EL integrity and thinning in CNV or disciform scar formation in AMD patients compared to age matched controls (Chong et al., 2005). Correspondingly, serum EDPs (Sivaprasad et al., 2005) and elastin antibody levels (Morohoshi et al., 2012) were also found to be significantly higher in wet AMD patients compared to the age matched controls. Moreover, increased lung elastin degradation in smokers has been linked to AMD progression; also the administration of smoke induced oxidized elastin peptides has exacerbated the AMD phenotype in older mice (Annamalai et al., 2020), further reaffirming the association of elastin turnover abnormalities to AMD. Aging-associated elastin turnover could be a contributing factor in AMD; however, other factors such as increased elastase secretion caused by pathological oxidative stress/inflammation might be involved in worsening the phenotypes that lead to AMD progression.

Another interesting question is why people suffering from other ocular diseases with reduced elastin fibers do not also develop AMD. One possible reason could be the tissue-specific degradation of elastic fibers in different ocular disease. For example, in the case of AMD, elastic fiber degradation at BrM appears to play a critical role in CNV progression, whereas in keratoconus elastin loss occurs at the cornea (Table 1). Local inflammation or oxidative stress in the outer retina during AMD can increase elastase secretion from RPE cells and infiltrating immune cells, contributing to BrM EL degradation/abnormal elastin turnover. CNV development corresponding to the BrM/EL damage reported in Sorsby's fundus dystrophy and Pseudoxanthoma elasticum PXE (Table 1), supports this observation in AMD. Reduced biomechanical properties of the BrM elastin layer and the inflammatory role of generated EDPs may contribute to AMD pathology, causing RPE atrophy and CNV, as postulated in Sorsby's and PXE.

5.4. Targeting the EDP-anti-elastin antibody-complement axis in a mouse model

Elevated serum levels of EDPs (Sivaprasad et al., 2005) and elastin IgG and IgM antibodies (Morohoshi et al., 2012), reported in early and neovascular AMD, led us to suggest that abnormalities in elastin homeostasis and the corresponding antibody production may play a role in AMD. Specifically, IgGs and/or IgMs that bind to ligands on cell surfaces, basement membranes, or extracellular matrices can participate in tissue damage involving either the engagement of the complement system (complement-dependent cytotoxicity; CDC) or Fcγ-receptor (FcγR) dependent cell-mediated cytotoxicity (antibody-dependent cell-mediated cytotoxicity; ADCC) (Saeed et al., 2017). Both of these mechanisms have been shown to participate in chronic, slowly progressing diseases (Natoli et al., 2018; Wang and Ravetch, 2015). We have previously shown that long-term smoke exposure in C57BL/6J mice leads to features of human dry AMD, including a loss in retinal function, thickening of BrM, loss of the elastin layer integrity as well as lipid deposition in the area of BrM, and these changes are dependent on the activity of the alternative pathway of complement (Kunchithapautham et al., 2014; Woodell et al., 2013, 2016). Hence, we speculated that immunizing C57BL/6J mice with elastin peptide oxidatively modified by cigarette smoke (ox-elastin) would augment pathology in smoke-exposed mice (Annamalai et al., 2020). As expected, immunization of mice with ox-elastin resulted in a large increase in anti-ox-elastin IgG and IgM antibodies, whereas, elastin immunization had a smaller effect. Importantly, and in agreement with our hypothesis, ox-elastin immunization led to exacerbated smoke-induced vision loss and thicker BrM and increased levels of IgM, IgG2b, IgG3, and complement activation products in the RPE/choroid when compared to controls. Based on the specificity of the antibodies produced, pathology could be driven by a mixed effect, with IgM and IgG3 antibodies activating complement leading to CDC, whereas IgG2b could engage FcγR on effector cells eliciting ADCC. CDC and ADCC involvement have been confirmed in mice in which the alternative pathway of complement (factor B, $fB^{-/-}$) or the Fcγ receptor ($Fc\gamma R^{-/-}$) were eliminated (Rohrer et al., 2021; Woodell et al., 2013). Despite not being conclusive as to the relative contributions of the two pathways being engaged by the anti-elastin antibodies, these results support the growing body of evidence linking oxidative stress, smoking, complement activation, and autoimmunity to AMD pathogenesis.

Peptide immunotherapy has been studied extensively for the treatment of various autoimmune diseases, allergies as well as cancers (Larche, 2014; Romano et al., 2019; Shakya and Nandakumar, 2018; Smith and Peakman, 2018). While the exact mechanisms of immune modulation and suppression of disease by peptide immunotherapy has not yet been fully defined (Sabatos-Peyton et al., 2010), peripheral tolerance towards certain antigens in laboratory animals is generated by repeated exposure to the antigen, and is thought to involve deletion of reactive T cells, the induction T cell activation and/or generation of a regulatory T (Treg) cell response, or an altered response of macrophages (Butcher et al., 2018). If the generation of EDPs and their corresponding antibodies were to be causally related to pathology in this model, one could argue that attenuation of disease with peptide immunotherapy should be possible. We tested this hypothesis in smoke-exposed mice injected with oxidized mouse elastin peptide over the course of smoke exposure (Annamalai et al., 2020). Importantly, animals treated with the low dose of oxidized

elastin peptide exhibited a reduced humoral immune response, producing lower levels of ox-elastin-specific IgG and IgM antibodies when compared to control smoke-exposed mice. This reduced level of serum anti-ox-elastin antibodies was associated with reduced levels of IgG/IgM deposition in RPE/choroid, and blunted complement activation. Structurally and functionally, low dose peptide immunotherapy resulted in preservation of BrM and improved contrast sensitivity compared to the non-tolerized mice. Finally, cytokine levels in the RPE/choroid fraction in response to treatment is suggestive of an induction of tolerance. Activation of the immune system by non-self cells or proteins (i.e., peptides leading to an immune response) involves a Th1 response driven by IFNγ; where as exposure to self cells or proteins, triggers tolerance, invloving a Th2 response activated by IL-4. Interestingly, peptide immunotherapy decreased IFN and increased IL-4. Our observations support the hypothesis by Nussenblatt and colleagues, that AMD might be suitable for tolerance therapy, which would re-align the adaptive immune response by suppressing T cell responses (Nussenblatt et al., 2014). Unfortunately, the smoke model in C57BL/6J mice does not lead to breakthrough choroidal neovascularization, such that the concept of tolerance for the prevention of CNV could not be tested.

6. Concluding remarks

Elastin and enzymes regulating its turnover play crucial roles in the pathogenesis of many ocular diseases including AMD, and especially neovascular AMD. BrM elastin turnover is critical in the pathogenesis of AMD. Degradation of, in particular, macular BrM elastin and thus reducing macular BrM integrity and its increasing porosity can aid the migration of choroidal endothelial cells into the subretinal space causing neovascularization. While the origin of the elastases breaking down the elastin layer is unclear, RPE and infiltrating immune cells are known to secrete proteases such as elastase, metalloproteinases, gelatinases, and their inhibitors (TIMPs 1, 2), and elastase activity was found to be increased with aging, contributing to the age-related degradation of BrM. Additionally, elastin fragments or EDPs generated by the elastin degeneration can have several cell-signaling roles; this includes EDP-mediated cell migration, differentiation, proliferation, chemotaxis, and angiogenesis. In endothelial cells in particular, EDPs induces angiogenesis via upregulating MMPs (MT1-MMP). Finally, we have proposed a mechanism whereby which EDPs act as ligands in the aged BrM, leading to the binding of anti-elastin antibodies, triggering complement activation. Elastin degradation, serum EDP levels, and anti-elastin antibody levels were increased in AMD patients compared to age matched healthy subjects, suggesting that abnormal elastin turnover reported in AMD is not just an age-related affect, but rather it can directly contribute to the pathology. It would be of great interest to further investigate the key roles played by proteases, oxidative stress, and inflammation in AMD pathogenesis. Insights gained from other conditions such as COPD suggest that elastase inhibitors should be explored as a new therapeutic approach. Altogether, the current evidence suggests that inhibition of elastin degradation could be a promising therapeutic strategy in neovascular AMD by preventing pathological angiogenesis and complement activation.

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Data availability

No data was used for the research described in the article.

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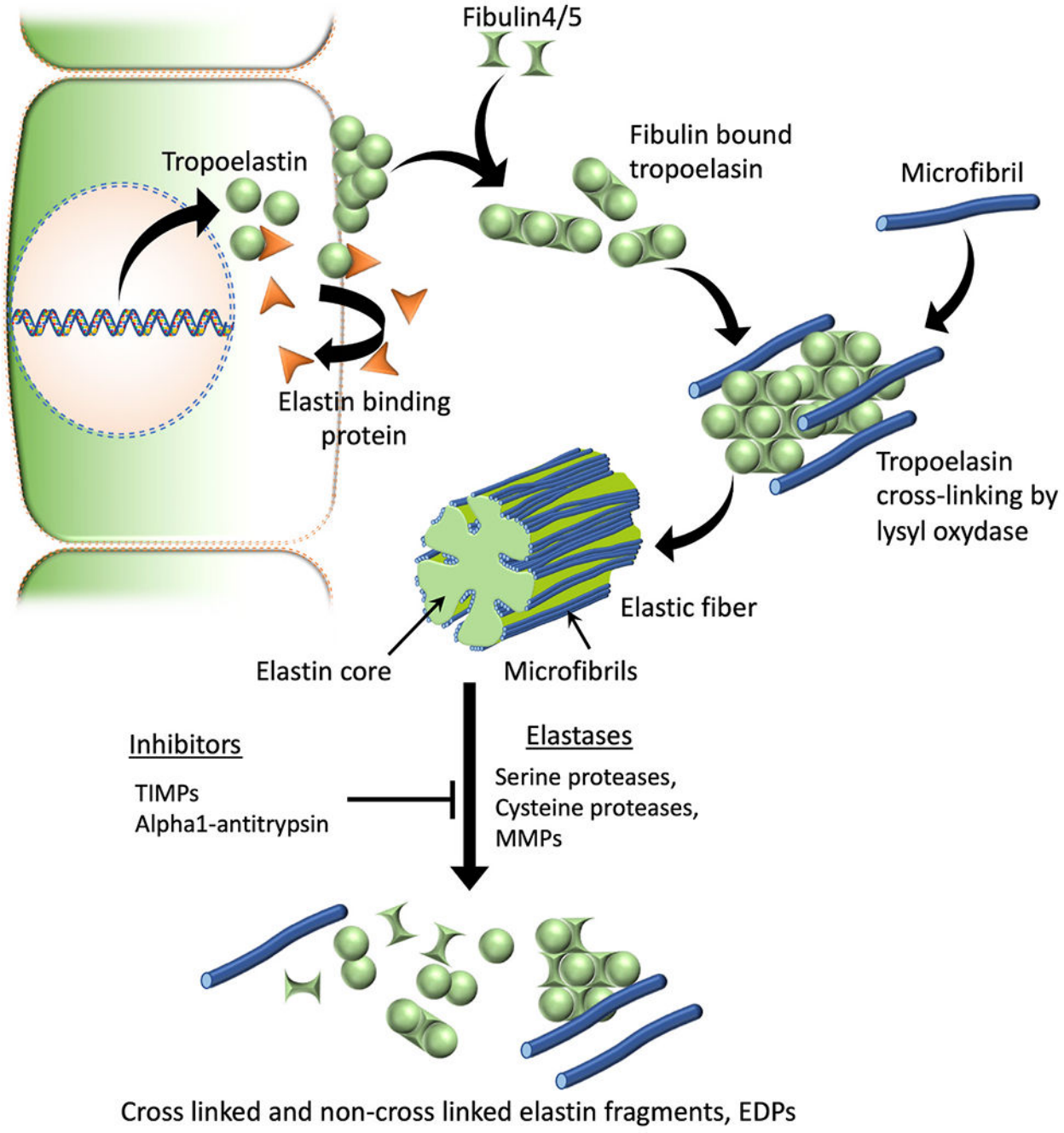

Fig. 1. Elastic fiber formation and degradation.

Tropoelastin monomers are formed intracellularly, which then will be chaperoned and transported to the ECM by elastin binding proteins. At the cell surface elastin binding proteins will be released leading to tropoelastin self-aggregation or coacervation. Coacervation leads to next stages of elastic fiber assembly such as binding of fibulins, microfibrillar deposition, recruitment of lysyl oxidase, elastin cross-linking and mature fiber formation. Serine proteases such as neutrophil elastase & HTRA1, cysteine proteases like cathepsins K, V, L, S & B, and several metalloproteases (MMPs 2, 3, 7, 9, 12 & 14)

are known to have elastolytic capability and can contribute to the degradation of insoluble elastic fibers to generate elastin fragments or EDPs. TIMPs and alpha-1 proteases such as alpha-1 antitrypsin are known elastase inhibitors that can prevent the degradation of elastic fibers.

Abbreviations: TIMPs, Tissue inhibitors of metalloproteinases; MMPs, Matrix metalloproteinases; EDPs, Elastin-derived peptides.

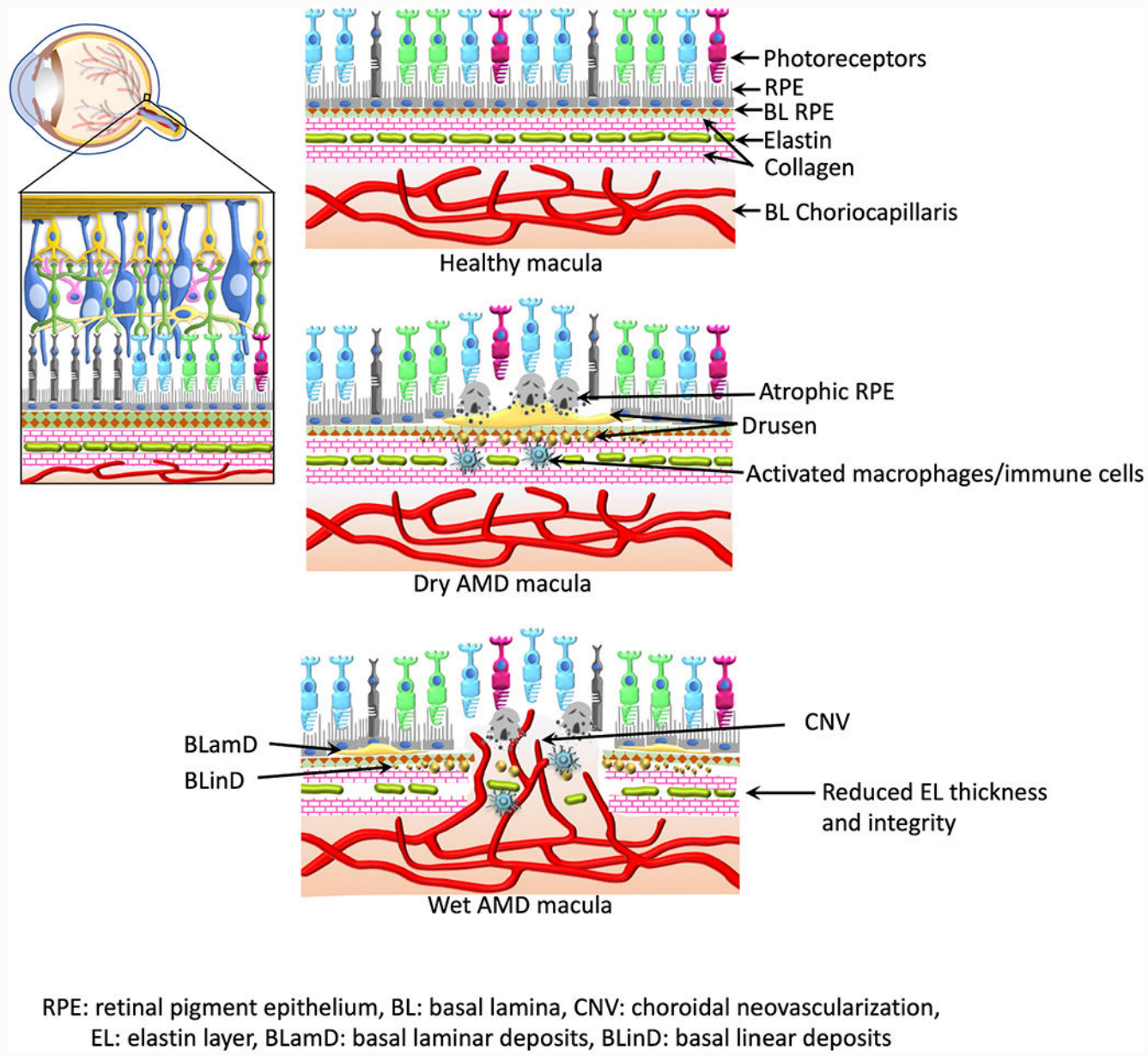

Fig. 2. Macular Bruch's membrane elastin layer degradation in AMD.

Figure indicates schematic representation of eyeball, retina, and macula during health and AMD. Reduced macular elastin layer integrity and thickness have been reported in AMD patients compared to the age matched healthy subjects; BrM elastin layer thinning, calcification, and porosity were more evident and were corresponding to the distribution of CNV lesions in wet AMD macula.

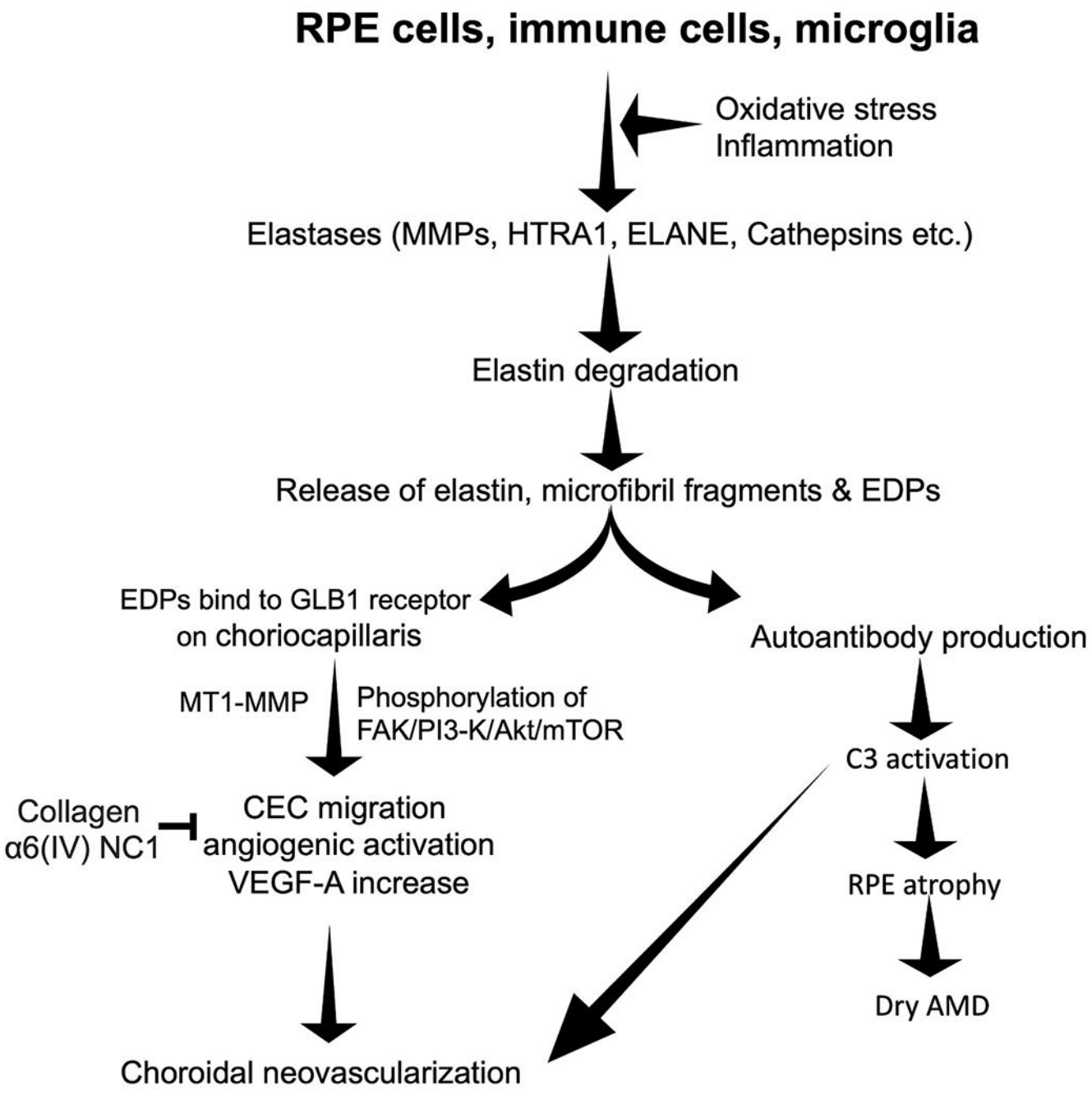

Fig. 3. Proposed mechanisms of elastin turnover in the pathogenesis of dry and wet AMD.

During AMD pathogenesis local inflammation or oxidative stress can upsurge the elastase secretion from RPE cells, infiltrating immune cells such as macrophages/neutrophils and microglia leading to elastin degradation and EDP generation. EDPs can bind to the GLB1 receptors present on the choroidal endothelial cells (Skeie et al., 2012). EDP binding to the endothelial cells can increase the MT1-MMP level (Robinet et al., 2005) and the phosphorylation of FAK/-PI3–K/Akt/mTOR (Gunda et al., 2013) pathway, leading to VEGF upregulation (Sounni et al., 2004), endothelial cell migration and pathological neovascularization. Collagen fragment α6(IV) NC1 can inhibit this elastin

peptide mediated endothelial cell migration and angiogenesis (Gunda et al., 2013). Products of elastin degradation can also increase the autoantibody production and complement C3 overactivation (Annamalai et al., 2020), suggesting its possible role in RPE death and dry AMD pathogenesis. Abbreviations: RPE, Retinal Pigment Epithelium; MMP, matrix metalloproteinase; HTRA1, High-Temperature Requirement A Serine Peptidase 1; ELANE, Neutrophil Elastase/leukocyte elastase; EDP, Elastin Derived Peptides; GLB1, Galactosidase beta 1 receptor; MT1 MMP, Membrane Type 1 MMP; Collagen α6(IV) NC1, type IV collagen α-6 chain-derived non-collagenous domain; VEGF-A, Vascular Endothelial Growth Factor A; FAK/-PI3–K/Akt/mTOR, Focal adhesion kinase(FAK)/-phosphoinositide 3-kinase/protein kinase B (PI3K/AKT)/mammalian target of rapamycin (mTOR).

Table 1

Role of elastin turnover in ocular diseases. Abbreviations: MMP, Matrix Metalloproteinase; LOX, Lysyl oxidase; BrM EL, Bruch's membrane elastin layer; CNV, choroidal neovascularization; TIMP, tissue inhibitors of metalloproteinases; EDP, elastin derived peptides; HTRA1, High-Temperature Requirement A Serine Peptidase 1; LoxL1, Lysyl oxidase like 1; POAG, primary open angle glaucoma. The arrows indicate the direction of change.

Disease	Pathological implications	References
Floppy eyelid syndrome	↓ tarsal elastin level, ↑ elastin degradation, ↑ MMP 7 and 9 levels	(Netland et al., 1994; Schlötzer-Schrehardt et al., 2005)
Keratoconus	↓ LOX distribution/activity in the cornea, ↓ elastic fiber system in the cornea	(Bykhovskaya et al., 2012; Dudakova and Jirsova, 2013; Dudakova et al., 2012; White et al., 2017b)
Ocular components of Marfan syndrome	↑ectopia lentis, ↑glaucoma, ↓ retinal/choroidal vascular health	(Di Marino et al., 2020; Nahum and Spierer, 2008; Zadeh et al., 2011)
Ocular components of Pseudoxanthoma elasticum	↑ Calcification and fragmentation of BrM EL, ↑ risk of CNV and macular atrophy	(Charbel Issa et al., 2009; Risseeuw et al., 2020b)
Sorsby's fundus dystrophy	↑ Subretinal deposits of mutated TIMP-3, ↑ BrM thickening and fragmentation of BrM EL, ↑CNV	(Anand-Apte et al., 2019; Chong et al., 2000)
Rhegmatogenous retinal detachment	↓ LOX activity and ↑ MMP 2 and 9 levels in the vitreous	(Coral et al., 2008)
Diabetic retinopathy	↑Neutrophil elastase levels and ↑vascular permeability, ↓ LOX activity and ↑ MMP 2 and 9 in the vitreous, ↑ serum levels of anti-elastin IgA antibodies	(Coral et al., 2008; Liu et al., 2019; Nicoloff et al., 2000)
AMD	↓ integrity of macular BrM EL, ↑ thinning of macular BrM EL, ↑ serum levels of EDPs, ↑ serum levels of elastin antibodies, ↑Elastin and HTRA1 gene polymorphisms in polypoidal choroidal vasculopathy (PCV) and AMD	(Chong et al., 2005; Dewan et al., 2006; He et al., 2021; Kondo et al., 2008; Morohoshi et al., 2012; Sivaprasad et al., 2005; Tong et al., 2010; Yamashiro et al., 2011; Yanagisawa et al., 2015)
Glaucoma (POAG)	↓LoxL1 and ↑elastin fragmentation/aggregate depositions at the lamina cribrosa, ↑Thickening of the elastic fiber sheath in trabecular meshwork, ↑elastin deposition in the Schlemm's canal endothelial cells	(Pena et al., 1998; Quigley et al., 1994; Schlötzer-Schrehardt et al., 2012; Tektas and Lütjen-Drecoll, 2009; Umihira, 1993; Umihira et al., 1994)

Table 2

List of enzymes with elastase activity. Abbreviations: VSMC, vascular smooth muscle cells; RPE, retinal pigment epithelium; MMP, matrix metalloproteinase; HTRA1, High-Temperature Requirement A Serine Peptidase 1.

Enzymes	Source	Mode of action	References	Presence in RPE		
				Human	Mouse	ARPE19
Neutrophil elastase (Ela-2)	Neutrophils, VSMCs	Serine protease	Cocciolone et al., 2018; Kim et al., 2011; Mustafi et al., 2013; Sharma et al., 2005; Zhang et al., 2012	✓		×
Cathepsin K, V	Neutrophils, VSMCs, Macrophages	Cysteine protease	Cocciolone et al., 2018; Yasuda et al., 2004; Mustafi et al., 2013; Sharma et al., 2005; Zhang et al., 2012	✓	✓	✓
Proteinase-3 (Pr-3)	Neutrophils	Serine protease	Cocciolone et al., 2018; Mustafi et al., 2013; Sharma et al., 2005; Zhang et al., 2012	✓		×
MMP-2	Macrophages, VSMCs, Glioma endothelial cells	Metallo proteinase	Ahir et al., 2002; Bandyopadhyay and Rohrer, 2012; Cocciolone et al., 2018; DeVera and Tosini, 2020; Mustafi et al., 2013; Sharma et al., 2005; Yu and Stamenkovic, 2000; Zhang et al., 2012	✓	✓	✓
MMP-3	Keratinocytes, Macrophages, Endothelial cells, VSMCs	Metallo proteinase	Alge-Priglinger et al., 2009; DeVera and Tosini, 2020; Murphy et al., 1991; Mustafi et al., 2013; Sharma et al., 2005; Sternlicht et al., 1999; Zhang et al., 2012	✓	✓	×
MMP-7	Macrophages, Leukocytes, Endothelial cells, Paneth cells	Metallo proteinase	Cocciolone et al., 2018; Heinz et al., 2011; Mustafi et al., 2013; Sharma et al., 2005; Taggart et al., 2005; Zhang et al., 2012	×		×
MMP-9	Macrophages, VSMCs, Neutrophils, Glioma endothelial cells	Metallo proteinase	Ahir et al., 2002; Bandyopadhyay and Rohrer, 2012; Cocciolone et al., 2018; Mustafi et al., 2013; Sharma et al., 2005; Taggart et al., 2005; Yu and Stamenkovic, 2000; Zhang et al., 2012	✓	✓	✓
MMP-12	Macrophages	Metallo elastase	Cocciolone et al., 2018; Hautamaki et al., 1997; Mustafi et al., 2013; Sharma et al., 2005; Taggart et al., 2005; Zhang et al., 2012	✓		×
MMP-14	Cardiac fibroblasts, Cardiomyocytes, VSMCs	Membrane-type metallo proteinase	DeVera and Tosini, 2020; Miekus et al., 2019; Mustafi et al., 2013; Rabkin, 2017; Sharma et al., 2005; Zhang et al., 2012	✓	✓	✓
Cathepsin L	Macrophages, VSMCs	Cysteine protease	Cocciolone et al., 2018; Mustafi et al., 2013; Sharma et al., 2005; Zhang et al., 2012	✓		✓
Cathepsin S	Macrophages, VSMCs	Cysteine protease	Cocciolone et al., 2018; Mustafi et al., 2013; Sharma et al., 2005; Sukhova et al., 1998; Zhang et al., 2012	✓		×
HTRA1	Müller cells, Mononuclear-phagocytes, Monocytes	Serine protease	Beguier et al., 2020; Dewan et al., 2006; Jones et al., 2011; Menon et al., 2019; Mustafi et al., 2013; Sharma et al., 2005; Vierkotten et al., 2011; Zhang et al., 2012	✓	✓	✓
Cathepsin B	Fibroblast like synoviocytes	Cysteine protease	Mustafi et al., 2013; Sharma et al., 2005; Taggart et al., 2005; Tong et al., 2014; Zhang et al., 2012	✓		✓
Cathepsin G	Neutrophils	Serine protease	Boudier et al., 1991; Mustafi et al., 2013; Sharma et al., 2005; Zhang et al., 2012	✓	✓	✓