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Bruch's Membrane: The Critical Boundary in Macular Degeneration

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1. Introduction

In the early second century A.D., the Roman Empire had reached its zenith. The *pax romana* extended to all the lands touching the Mediterranean, counterclockwise from Northern Africa to Palestine, through Asia Minor, Gaul and Hispania, all the way to modern Wales and England. This last province of Britannia was visited by the emperor Hadrian, where he put on a show of force in response to a recent uprising. Most famously, during this visit Hadrian ordered the construction of a set of earthworks that now bears his name, stretching nearly eighty miles from Carlisle to Newcastle. Studded with fortifications, and manned with highly trained foreign legionnaires, this imposing structure stretched the width of the island and its ruins are impressive still today (Figure 1). While there is disagreement about the principal purpose of Hadrian's Wall—whether primarily as a symbolic marker of the northern extent of the Empire, a defensive fortification, or as a means of regulating commerce—the Historia Augusta states that the wall was constructed *qui barbaros Romanosque divideret;* to separate the Romans from the Barbarians(Magie, 1921).

Whatever the principal motivation for its construction, Hadrian's wall did stand at the frontier between the Roman controlled territory and the Scots, Picts and other uncontrollable northern tribes of reputed violence. To the mind of the most Romanized Britons, who never met one of their northern neighbors, the wall must have represented the safe barrier between civilization and chaos.

Like the boundary between the civilized and barbarian world, a similar barrier stands at the threshold between the neural retina and the blood. The photoreceptor cells of the human retina face a dilemma. These cells are extremely energetic, consuming large quantities of oxygen. Elegant physiological studies by Linsenmeier and colleagues have shown that the oxygen tension from the RPE to the outer nuclear layer plunges over a distance of about 50μm, and that consumption at the level of the inner segment is dramatic(Birol, et al., 2007). Thus, the outer retina has a requirement for a large vascular supply. This requirement is met by the choriocapillaris, a unique vascular bed beneath the RPE. The endothelial cells (EC) of the choriocapillaris, like other EC, appear to use very little of the oxygen they deliver, instead likely relying largely on glycolytic metabolism. The choroid receives the vast

majority of the uveal blood supply (in some studies choroidal blood flow was estimated at more than 20x higher than in neural retina(Alm and Bill, 1973)). The proximity of the photoreceptor cells to the choroid is also required to remove wastes from the retina. Each RPE cell turns over many thousands of rod outer segment discs each day(Young, 1971) and this waste material from the RPE must be removed by the vasculature.

However, the juxtaposition of the choroid and retina poses a dilemma: while on one hand the photoreceptor cells have a major vascular requirement, on the other hand the microenvironment of the subretinal space is exquisitely regulated and the presence of subretinal fluid not inherent to this space (i.e., rhegmatogenous retinal detachment) results in rapid and severe vision loss. Having a dense vascular supply adjacent to a tissue that can not tolerate alterations in its interstitial space appears to be a recipe for disaster.

This dilemma is solved through the presence of soluble factors, such as PEDF(Dawson, et al., 1999; Ohno-Matsui, et al., 2001) and insoluble (structural) factors that keep the vasculature in check. In this chapter we will first briefly review the disease age-related macular degeneration (AMD) and then discuss the (normally) insoluble factors that guard the retina against vascular intrusion, namely Bruch's membrane (BrM), that play a role in preserving central vision.

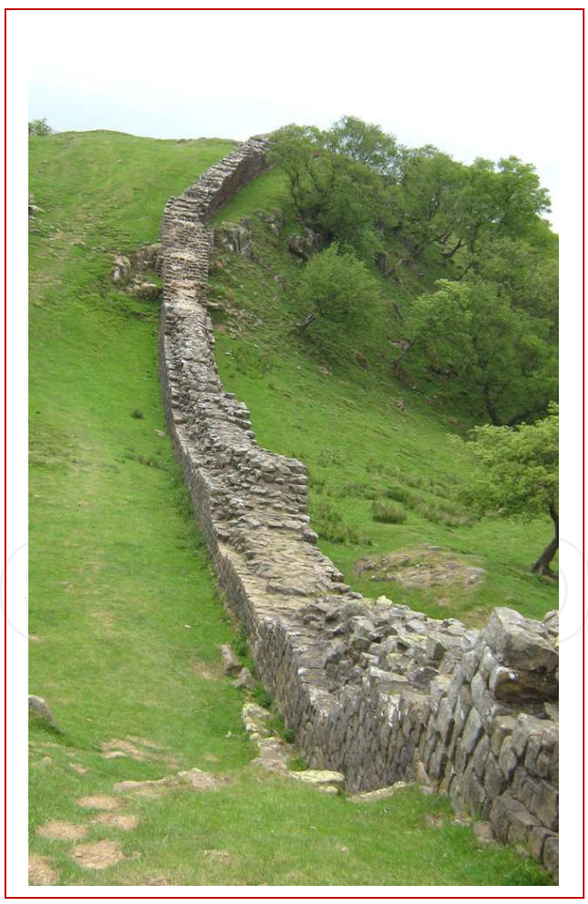

Fig. 1. Section of Hadrian's wall near Carlisle, Cumbria, UK. While sections of stone have been plundered over the hundreds of years of its existence, the wall continues to have an imposing presence. Photo courtesy of Jenna M. Mullins.

2. Clinical relevance of Bruch's membrane dysfunction: Age-related macular degeneration

The most common ocular condition of elderly humans with BrM dysfunction is age-related macular degeneration (AMD). This disease affects the center of the vision by damaging the macula, the critical portion of the retina responsible for fine visual acuity and daily activities such as reading, driving and recognizing faces. It is the most common cause of blindness in the elderly population in the western world(Klein, et al., 1992; Mitchell, et al., 1995; Vingerling, et al., 1995). Genetic variants conferring risk for developing AMD include mutations in complement factor H (*CFH*), complement component 2 (*C2*), *C3, CFB*, and the age-related maculopathy susceptibility 2/HtrA serine peptidase 1 (*ARMS2/HTRA1*)(Bergeron-Sawitzke, et al., 2009; Edwards, et al., 2005; Fritsche, et al., 2008; Hageman, et al., 2005; Haines, et al., 2005; Kanda, et al., 2007; Klein, et al., 2005; Maller, et al., 2007; Rivera, et al., 2005; Yates, et al., 2007); also reviewed in this volume (Dubielecka and Hoh). As AMD is a complex disease, genetic variation accounts for only a portion of risk; environmental influences also play an important role. The most consistently demonstrated factor conferring environmental risk is smoking (Chakravarthy, et al., 2007; de Jong, 2006).

Drusen, focal circular deposits typically located in the macula, are the most commonly identified clinical feature of early AMD. They vary in size and distribution but are usually symmetric between the two eyes. These deposits, along with pigment alterations in the RPE (Figure 2A), increase the risk for development of more advanced stages of AMD associated with visual loss: geographic atrophy (GA) and choroidal neovascularization (CNV) (Figure 2B). Geographic atrophy is the default end-stage of vision loss due to AMD when CNV does not develop. It is recommended that patients with high-risk features (i.e. several medium to large drusen, RPE changes, and or CNV in the fellow eye) take age-related eye disease study (AREDS)-formula vitamins to decrease the chance of CNV.(2001)

Geographic atrophy (GA) refers to a well-circumscribed area of RPE and photoreceptor cell loss in the macula associated with loss of retinal function that correlates clinically to dark spots (scotomas) in the vision. When involving the fovea, visual acuity declines depending on the size of the area affected. Visual loss from GA is indolent, but once present rates of GA progression occur up to 1.52 mm^2/yr to 3.02 mm^2/yr(Holz, et al., 2007; Lindblad, et al., 2009; Sunness, et al., 2007). There is currently no effective treatment for GA but several clinical trials are underway.(Meleth, et al., 2011)

In contrast to GA, CNV is associated with more acute vision change that often presents to the ophthalmologist with metamorphopsia, scotoma, and/or decreased vision. Clinical signs include subretinal hemorrhage, lipid exudate, fluid, and a grey-green lesion (Figure 2B). Classically, dye leakage on fluorescein angiography confirms the growth of the new vessels that occur in the subRPE or subretinal space (Figure 2C). Optical coherence tomography (OCT) characterizes the presence of subretinal fluid, intraretinal edema, RPE detachment, or subRPE/retinal tissue from CNV (Figure 2D,E). Intravitreal injections of anti-VEGF agents are first-line therapy for most CNVs presenting to the retina specialist and almost always stabilize, if not improve, visual acuity.(Brown, et al., 2006; Martin, et al., 2011; Rosenfeld, et al., 2006; Tufail, et al., 2010) Because of its reliability and reproducibility, OCT is used to monitor the CNV and often guides the clinician in the decision-making process for long-term treatment.(Fung, et al., 2007)

The pathologic correlates of BrM changes in AMD is discussed further below. The next sections review the anatomy and early-onset consequences of changes to BrM.

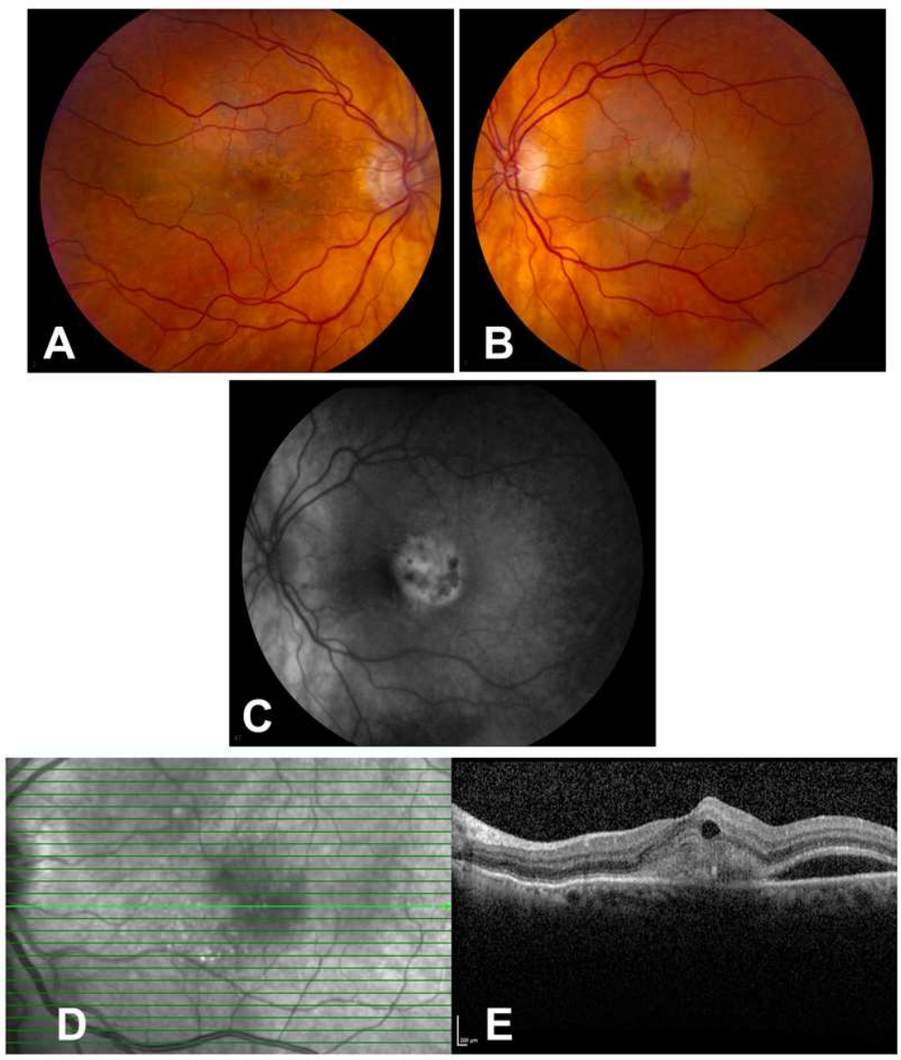

Fig. 2. 87 year-old female presented with a one week history of metamorphopsia in the left eye and visual acuity of 20/80. (A) Fundus photo of the right eye demonstrating non-neovascular AMD. There are two medium sized drusen temporal to the fovea with few small drusen in the superotemporal arcade. RPE changes are most prominent just superior and nasal to the fovea. Visual acuity in this eye was 20/25. (B) The macula of the left eye has a deep gray-green lesion with subretinal hemorrhage consistent with neovascular AMD. (C) Fluorescein angiogram confirms leakage of the CNV centered in the temporal macula but involving the fovea. (D) Infrared image signifying the horizontal scan position of the spectral-domain OCT image of the left eye (E) through the fovea shows subfoveal material, intraretinal edema with a cyst along the temporal edge of the fovea and temporal subretinal fluid.

3. Bruch's membrane: Anatomy

The name "membrane" referring to a refractive band on histological sections has a long history in ophthalmology, and includes the membranes of Descemet and Bowman, as well as the descriptive internal and external limiting membranes. Since the invention of the transmission electron microscope, the term membrane became applied to much smaller structures (plasma membrane, nuclear membrane and basement membrane).

BrM itself is a multilayered, extracellular matrix compartment that includes two basement membranes (more appropriately called basal laminae) at its inner and outer aspects (Figure 3). In addition to the basal laminae of the RPE and choriocapillaris, BrM possesses a central layer of elastin surrounded by two layers of collagen. In normal physiology, BrM appears to function as a robust barrier against neovascularization.

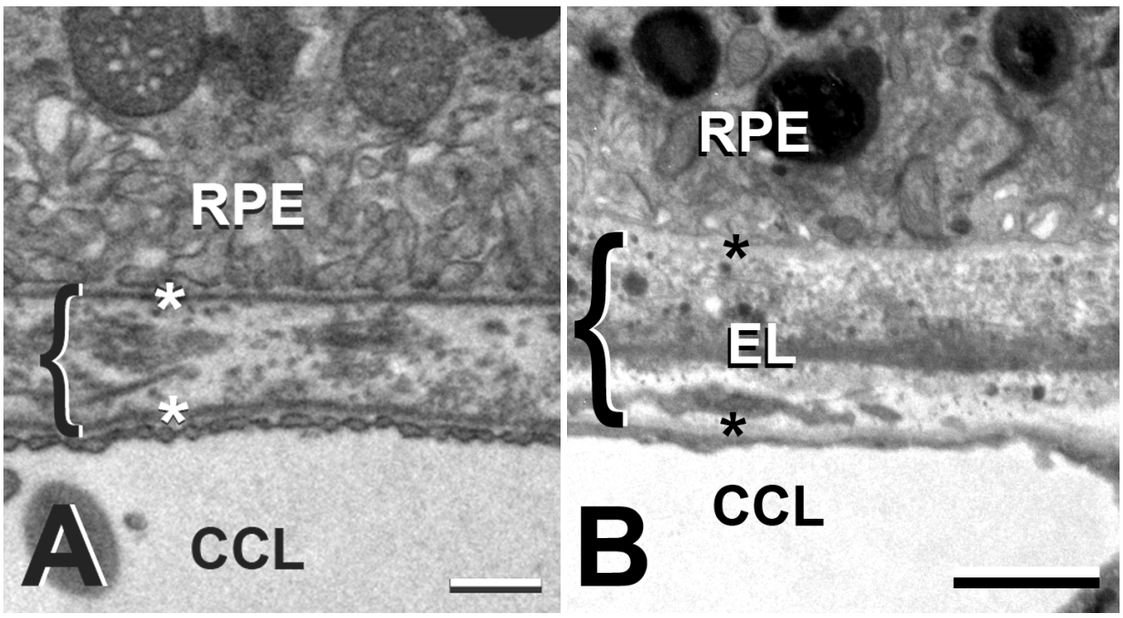

Fig. 3. Ultrastructure of murine (A) and human (B) Bruch's membrane. The basal laminae of the RPE (top) and choriocapillaris (bottom) are indicted by asterisks. Scale bar = 500nm (A) and 2µm (B). Labels indicate Bruch's membrane (bracket), the basal laminae (asterisks), the elastic lamina (EL), and RPE cell and the choriocapillaris lumen (CCL). Scale bar = 2µm. Left panel

Apart from its structural support function, BrM is also highly permeable to fluid and small molecules like oxygen and glucose. This quality likely derives from its sieve like structure appreciated by *en face* electron microscopy(Hogan, et al., 1971). Tracer studies in animals show that BrM (as well as the fenestrated choriocapillaris(Pino and Essner, 1981)) restrict the passage of even modestly sized proteins, while *in vitro* studies of excised human BrM can allow passage of molecules 200kDa or larger(Moore and Clover, 2001), recently discussed(Hussain, et al., 2010). Studies of hydraulic conductivity have invariably shown that the permeability of BrM decreases with increasing age (see below). RPE derived vascular endothelial growth factor also passes through BrM where it influences the structure and function of the choriocapillaris(Saint-Geniez, et al., 2009).

Thus, in normal physiology, there is ready transport of water, nutrients, metabolites, retinoids, and other molecules necessary to maintain retinal health and function in both directions.

4. Classes of Bruch's membrane molecules and their expression

BrM is bounded by two basal laminae (belonging to the RPE and choriocapillaris respectively), which contain the normal array of basal lamina constituents (collagen type IV, laminin, heparan sulfate proteoglycan, and entactin/nidogen)(Kunze, et al., 2010). The specific collagen IV isoforms present within human(Chen, et al., 2003) and mouse(Bai, et al., 2009) eyes differ between the RPE and choriocapillary basal laminae.

Between the two basal laminae, two layers of fibrillar collagens, consisting of types I and III collagen, surround a central core of elastin.(Das, et al., 1990; Nakaizumi, 1964; Nakaizumi, et al., 1964b). The elastic layer, when viewed en face, shows a crisscrossing pattern of web-like fibers with spaces to permit diffusion and allow deformability. This layer has received attention due to its alterations in pathology and biological activity of its degradation products (see below). Elastic fibers in other tissues bind transforming growth factor beta(Karonen, et al., 1997) and endostatin(Miosge, et al., 1999), and a similar association of anti-angiogenic proteins with BrM elastin may therefore "repel" vascular growth into the subretinal space when the elastic layer is intact and when the stimulus for neovascularization is not overwhelming. It is easy to envision that loss of elastin, by whatever mechanism, could lead to removal of a structural and biochemical barrier against choroidal neovascularization. Elastin may also protect against complement mediated injury through its association with the complement regulator decay accelerating factor/CD55(Werth, et al., 1988).

In addition, numerous other ECM molecules have been noted in BrM including the anti-angiogenic glycoprotein thrombospondin(Uno, et al., 2006) and sulfated proteoglycans(Call and Hollyfield, 1990; Clark, et al., 2011; Hewitt, et al., 1989). These molecules likely play important roles in hydration of the matrix, contribute to its barrier function(Prunte and Kain, 1995), the sequestration of growth factors, and may interact with the complement system (see below).

5. Genetic variations affect Bruch's membrane and cause early-onset macular dystrophies

The crucial role of ECM proteins in the macula is underscored by the fact that Bruch's membrane in this region is especially susceptible to mutations in genes with structural or regulatory roles in ECM metabolism. We briefly review a few of these familial macular diseases (i.e. macular dystrophies) with ECM abnormalities below.

Mutations in fibulins: Autosomal dominant radial drusen (also known as Malattia Leventinese, Doyne honeycomb retinal dystrophy, and dominant drusen) is a rare condition caused by a mutation (Arg345Trp) in the *EFEMP1* gene(Stone, et al., 1999). This gene encodes the ECM protein fibulin-3. Patients with this mutation typically have histologically unusual drusen like deposits that radiate from the macula, and may be complicated by neovascularization and/or atrophy(Michaelides, et al., 2006). The fibulin-3 protein is

localized to some BrM deposits, as well as photoreceptor outer segments and retinal ganglion cells(Marmorstein, et al., 2002). Mice harboring one or more copies of the mutant *Efemp1* allele develop subRPE deposits resembling basal laminar deposit in human eyes(Fu, et al., 2007; Marmorstein, et al., 2007). In addition to the gene that encodes fibulin-3, mutations in the fibulin-5 gene, that also alter the secretion of the mutant protein(Lotery, et al., 2006), are associated with sporadic cases of AMD(Stone, et al., 2004). This protein regulates elastin assembly(Nakamura, et al., 2002; Yanagisawa, et al., 2002) and is localized to aging BrM and basal deposits in AMD eyes(Mullins, et al., 2007). Notably, fibulin-3 is a binding partner of tissue-inhibitor of metalloproteinase-3 (discussed more fully below)(Klenotic, et al., 2004), serving as a mechanistic link for neovascular membranes through TIMP-3 dysregulation in both conditions. Both fibulin-3 and fibulin-5 are widely expressed, and the reason for macular specific disease—and the pattern of drusen seen with EFEMP1 mutation—remains to be determined. Interestingly, the normal fibulin-5 protein inhibits angiogenesis, and its perturbation could remove a block to pathologic neovascularization(Albig and Schiemann, 2004).

Mutations in TIMP3: Another macular dystrophy, Sorsby fundus dystrophy, is caused by any of several mutations in the tissue inhibitor of metalloproteinases-3 gene, *TIMP3*(Weber, et al., 1994). Like fibulin-3, the TIMP3 protein is localized to BrM and is a major component of drusen(Fariss, et al., 1997). TIMP3 is capable of inhibiting VEGF-mediated angiogenesis by blocking the binding of VEGF to VEGF receptor-2 thereby inhibiting downstream signaling.(Qi, et al., 2003) Histopathology of eyes with Sorby fundus dystrophy shows dramatically thick, confluent deposits with a fragmented elastin layer of BrM (Capon et al., 1989; Chong et al., 2000). Bilateral subretinal neovascular membranes are frequently observed in patients with TIMP3 mutations by the fourth to fifth decades of life(Sivaprasad, et al., 2008; Sorsby and Mason, 1949). The most obvious explanation for grossly abnormal ECM deposits, as well as BrM breaks that permit neovasculrization, is that the TIMP-matrix metalloproteinase balance is disrupted, as TIMP3 substrates are metalloproteinases(Apte, et al., 1995). Interestingly, however, at least for one common TIMP3 mutation (Ser156Cys), the inhibition of MMP activity is not impaired compared to wild type TIMP3(Fogarasi, et al., 2008). Mouse models of SFD with dominant mutations have been generated and these animals show increased deposition of material at the level of BrM(Weber, et al., 2002).

Like the fibulins, TIMP3 is widely expressed(Apte, et al., 1994) and the reason for ocular specific disease is not clear, although it may relate to the unique relationship between the retina and choroidal vasculature. It is also notable that, in contrast to dominant mutations, mice that lack TIMP3 develop abnormal choroidal vasculature, and show loss of intercapillary pillars with large, sinusoidal vessels, indicating that TIMP3 normally functions in the development of the choroidal vasculature(Janssen, et al., 2008). Finally, it is notable that in a very large genome wide association study, an AMD susceptibility locus has been mapped to a region of chromosome 22 near the *TIMP3* gene(Chen, et al., 2011); the functional impact of the variants at this locus are yet to be explored.

Mutations in ABCC6: Whereas EFEMP1 and TIMP3 mutations have not as yet been associated with any pathology outside of the eye, mutations in *ABCC6* cause the systemic elastin disease, pseudoxanthoma elasticum (Bergen, et al., 2000; Le Saux, et al., 2000; Struk, et al., 2000).This disease affects elastic fibers in the skin and vasculature, in addition to BrM. The histopathologic phenotype shows breaks in BrM and elastin calcification and

fragmentation(Hagedoorn, 1939). The process of elastin calcification probably makes the brittle BrM susceptible to the fissures referred to clinically as angioid streaks, through which choroidal blood vessels invade the retina.

Thus, genetic conditions that affect the biochemistry of BrM can lead to a phenotype similar to end stage AMD.

6. Bruch's membrane aging changes

BrM undergoes age-related alterations that include anatomical, functional and molecular changes. We briefly review these below.

Ultrastructural changes: Anatomical changes in aging human BrM have been documented extensively and will not be covered to any significant degree here (see for example.(Feeney-Burns and Ellersieck, 1985; Hogan and Alvarado, 1967; Hogan, et al., 1971) These changes include the accumulation of membranous debris on both sides of the elastic lamina(Feeney-Burns and Ellersieck, 1985), accumulation of focal drusen between the RPE basal lamina and inner collagenous layer of BrM(Sarks, et al., 1999), and accumulation of more confluent deposits. These deposits may occupy the same space as drusen and exhibit the appearance of membranous debris (termed basal linear deposits) or may develop between the RPE cell and its basal lamina and have an amorphous or banded structure with a periodicity consistent with that of type VI collagen (referred to as basal laminar deposits)(Curcio and Millican, 1999; Knupp, et al., 2002; Nakaizumi, et al., 1964a; Sarks, et al., 2007). Changes in the elastic lamina are in their own section below.

Functional changes: In addition to the structural alterations that occur in BrM during aging, the function of BrM as a conduit for the passage of material between the RPE and choriocapillaris becomes substantially reduced. Functional changes in aging have been measured primarily through the use of Ussing chambers in which flow of tracer molecules can be assessed. A significant decrease in hydraulic conductivity—a measure of the permeability of tissue—has been described in the aging BrM(Hussain, et al., 2002; Moore, et al., 1995; Starita, et al., 1996). Hydraulic conductivity drops exponentially with advancing age, with greater than 50% reduction occurring every 20 years of age(Starita, et al., 1996). Sequential ablation of different layers of BrM suggests that the major site of resistance that differs between young and old eyes is the inner collagenous layer(Starita, et al., 1997) which also accumulates substantial debris in the aging macula(Newsome, et al., 1987b).

One probable function of elastin in BrM and the choroid is to structurally support this crucial tissue through the approximately 100,000 cycles of the choroidal pulse each day. Notably, in addition to loss of transport facility across BrM with age, a decline in the elasticity (approximately 1% per year after age 21) has also been reported(Ugarte, et al., 2006).

7. Molecular changes in Bruch's membrane also occur during aging

Lipids: An increased lipid content has been demonstrated using a variety of histochemical(Pauleikhoff, et al., 1990) and biochemical(Sheraidah, et al., 1993) approaches. Lipid content of BrM varies regionally between macular and extramacular samples(Gulcan, et al., 1993; Holz, et al., 1994).

Photoreceptor cells are extremely enriched for some classes of lipids, including the omega-3 fatty acid docosahexaenoic acid (DHA). This molecule can be metabolized to the neuroprotective molecule NPD1(Bazan, 2005) or oxidized to form a reactive, proinflammatory, angiogenic mediator carboxyethylpyrrole (CEP)(Hollyfield, et al., 2008). At least in the case of CEP, which is deposited in BrM in AMD(Gu, et al., 2003), photoreceptor outer segments are the most likely source. The photoreceptor origin of BrM lipids has been advanced for other classes as well(Pauleikhoff, et al., 1994). Some classes of lipids, including esterified and unesterified cholesterol(Haimovici, et al., 2001; Rudolf and Curcio, 2009), are assembled into distinct lipoprotein complexes and secreted by the RPE(Li, et al., 2005; Malek, et al., 2003; Wang, et al., 2011; Wang, et al., 2009). The provenance of all classes of lipids that accumulate in aging BrM is not entirely resolved, however, as recently reviewed(Ebrahimi and Handa, 2011).

Membrane attack complex of complement: In addition to lipidic changes, activation of the complement system has received considerable attention, particularly in view of the compelling genetic evidence that polymorphisms in the complement inhibitor complement factor H (*CFH*) (in addition to other genes whose products regulate the complement system) (Bergeron-Sawitzke, et al., 2009; Edwards, et al., 2005; Fritsche, et al., 2008; Hageman, et al., 2005; Haines, et al., 2005; Kanda, et al., 2007; Klein, et al., 2005; Maller, et al., 2007; Rivera, et al., 2005; Yates, et al., 2007) is strongly associated with AMD (discussed above). Studies on the membrane attack complex of complement show that the majority of labeling is found in BrM and particularly around the choriocapillaris(Gerl, et al., 2002; Hageman, et al., 2005; Mullins, et al., 2011b; Seth, et al., 2008; Skeie, et al., 2010). In one study, this labeling was found to be statistically higher in aged ($\geq$69) as compared to young ($\leq$56) donors, indicating an increased complement load during aging(Seth, et al., 2008). The aberrant activation of the complement system may result in endothelial cell loss in the choriocapillaris(Mullins, et al., 2011b), increased VEGF synthesis by the RPE(Nozaki, et al., 2006), upregulated ICAM-1 RNA and protein by the choriocapillaris(Skeie, et al., 2010), and abnormal T cell activation(Liu, et al., 2011). The pathology that follows these challenges by the RPE and choroid are likely to worsen with aging. While there is generally not strong histological evidence that the membrane attack complex complement places a significant challenge to the RPE, it is noteworthy that expression of CD46 decreases in the pathogenesis of geographic atrophy(Vogt, et al., 2011). Since CD46 is a negative regulator of complement activity, playing a similar function at the cell surface that CFH plays in the extracellular space, even small or histochemically undetectable levels of complement could injure the RPE in this weakened state.

In addition to age, genotype of *CFH* appears to regulate the amount of membrane attack complex in the BrM/choriocapillaris. In a small study of genotyped eyes, we found that donors homozygous for the high risk allele (H at codon 402) had on average over 50% more membrane attack complex than age matched donors who were homozygous for the low risk allele(Mullins, et al., 2011a). Thus both genotype and age contribute to the deposition of potentially cytolytic membrane attack complexes in the aging choroid.

Extracellular matrix: ECM structural molecules also show age- and AMD-related changes. We will briefly review these changes, with special emphasis on elastin. Studies in mice show increased deposition of basal lamina proteins in BrM with age(Kunze, et al., 2010). While drusen themselves contain few classic extracellular matrix molecules(Hageman, et al., 1999;

Newsome, et al., 1987a) aging BrM may show increased type IV collagen that contributes to its thickening(Marshall, et al., 1994). Basal lamina constituents are also present in granular basal laminar deposit material(van der Schaft, et al., 1994). In eyes with early AMD, the abundance of thrombospondin has been shown to be decreased(Uno, et al., 2006)—in view of the role of this protein in suppressing angiogenesis, its reduction may create a permissive environment for neovascularization. The altered homeostasis of ECM observed in aging and AMD may be in part due to the age-dependent reduction in matrix metalloproteinase activity(Guo, et al., 1999) and in the available pool of MMPs(Kumar, et al., 2010) that has been observed in human eyes. Proteomic studies of combined BrM/choroid layers from human donor eyes further reveal increases in some matrix proteins (e.g., MMP3, collagen I) and decreases in other proteins (e.g., nidogen-2, fibulin-1) during the progression of AMD(Yuan, et al., 2010).

Other age-related changes include the accumulation of iron and advanced glycation endproducts in BrM. While outside the scope of this article, the reader is referred to several excellent articles and reviews(Glenn, et al., 2009; Handa, et al., 1999; He, et al., 2007; Wong, et al., 2007), including in the current volume (Kaji et al.).

Elastin metabolism in AMD: Elastin is a hydrophobic glycoprotein of approximately 72kDa. It has an unusual primary sequence in which about one third of its amino acid composition is comprised of glycine. Unlike fibrillar collagens, which also contain glycines at every third amino acid, elastin does not form into triple helices. Instead, elastin monomers (tropoelastin molecules) are assembled into cross-linked networks by enzymatic modification of lysine residues by lysyl oxidases (Figure 4). The resulting network of hydrophobic elastin polymers is responsible for the elastic recoil of arteries, and indeed participates in maintenance of diastolic blood pressure(Faury, 2001). Elastin is found in multiple tissues including skin, large blood vessels, and BrM of the eye.

Several studies suggest that abnormal elastin physiology is involved in the pathogenesis of AMD, and especially neovascular AMD. One of the first indications of widespread elastin changes came from studies linking dermatologic changes with neovascular AMD. Whereas elastotic degeneration of sun-exposed skin is a typical finding, Blumenkranz and colleagues reported histopathologic evidence of elastotic degeneration of sun-protected skin in patients with CNV(Blumenkranz, et al., 1986). Second, Spraul and Grossniklaus, in performing morphometric studies on a series of human donor eyes with neovascular AMD, noted that calcification and fragmentation of the elastic lamina was a frequent finding(Spraul and Grossniklaus, 1997; Spraul, et al., 1999) suggesting elastin breakdown within BrM as a cause or consequence of AMD pathology. Third, Chong et al. made similar findings at the ultrastructural scale, in which we observed a relative paucity of macular elastin and noted that eyes with neovascular AMD tended to have a thinner and more porous elastic lamina of BrM than controls(Chong, et al., 2005) The thinning of the elastic lamina is in contrast to the general "thickening" of BrM during aging (which is actually accumulation of debris rather than expansion of its matrix components)(Feeney-Burns and Ellersieck, 1985). Fourth, genetic variations in genes associated with elastin biology are associated with macular disease, including *ABCC6* and *FBLN5* (discussed above) and polymorphisms in the elastin gene (*ELN*) that have been described in association with polypoidal choroidal vasculopathy(Kondo, et al., 2008). Mice deficient for the elastin crosslinking protein LOXL1 show disrupted BrM elastin and increased severity of experimental neovascularization(Yu,

et al., 2008). In addition, significantly elevated serum levels of elastin derived peptides (EDPs) have been found in the serum of CNV patients, with higher levels in CNV than in dry AMD, and higher levels in dry AMD than controls(Sivaprasad, et al., 2005). In this study, the authors noted that a sustained elevated level of EDPs could not be solely due to BrM degradation, and is instead likely to be due to systemic elastin abnormalities, consistent with those described by Blumenkranz et al. Finally, the breakdown products of elastin itself (elastin derived peptides) induce some angiogenic behaviors of choroidal endothelial cells(Skeie and Mullins, 2008)(Figure 4). Thus, the breakdown of elastin in BrM may simultaneously (a) remove a critical structural and chemical barrier to neovascularization and (b) actively induce the growth of pathologic blood vessels into the retina. Taken together, these studies provide strong support for the notion that abnormalities in elastin metabolism, both in ocular and extraocular tissues, are associated with the pathogenesis of neovascular AMD.

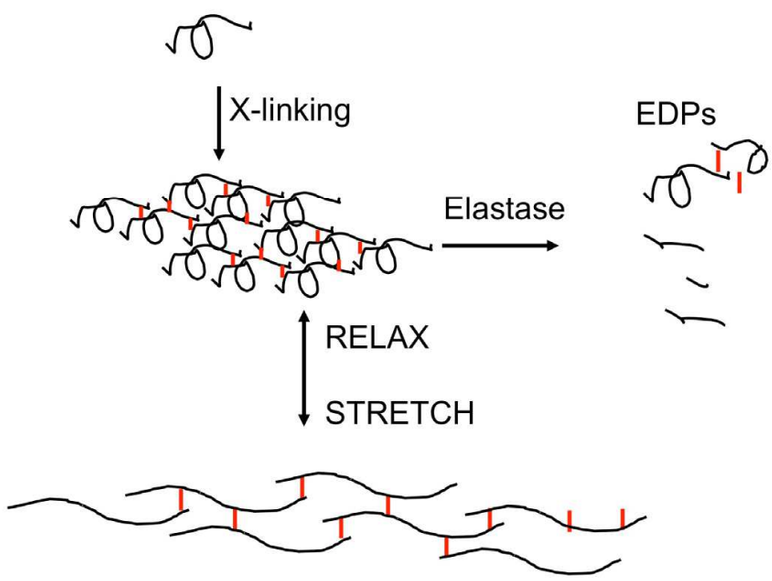

Fig. 4. Elastin monomers are crosslinked by lysyl oxidases into insoluble elastic networks that can repeatedly stretch and relax, essential for tissues like the choroid. Degradation of elastin by matrix metalloproteinases/elastases leads to the loss of integrity of the elastin network and the release of biologically active elastin derived peptides (EDPs) which can promote neovascularization. Adapted from Alberts et al., 2008

8. Consequences of ECM abnormalities

The development of either excessive material in Bruch's membrane or erosion of Bruch's membrane may have serious consequences whether congenital or age-related. Some events, such as membrane attack complex deposition, may lead to choroidal endothelial cell loss(Mullins, et al., 2011b) and place ischemic stress on the oxygen hungry photoreceptor cells. Vascular loss may exacerbate the development of drusen, as the ability of the choroid to remove debris is further compromised; in this context it is notable that drusen form preferentially in eyes and in regions of eyes depleted of capillary lumens(Lengyel, et al., 2004; Mullins, et al., 2011b; Sarks, et al., 1999)(Figure 5).

In addition, the interposition of lipid rich material between the retina and its vascular supply, even with a healthy choriocapillaris, can compromise the normal trafficking between these tissues(Curcio, et al., 2010). The accumulation of lipids in BrM (consistent with the reduced ability to move water through the aging BrM--discussed above) has led some investigators to the attractive hypothesis that normal pumping of fluid through the RPE, combined with a nonpermeable BrM, could cause pigment epithelial detachments(Pauleikhoff, et al., 1990). Moreover, the molecules that accumulate in aging Bruch's membrane—including CEP-modified proteins and advanced glycation endproducts—may themselves be toxic, pro-inflammatory, and pro-angiogenic(Ebrahem, et al., 2006; Glenn, et al., 2009; Ma, et al., 2007).

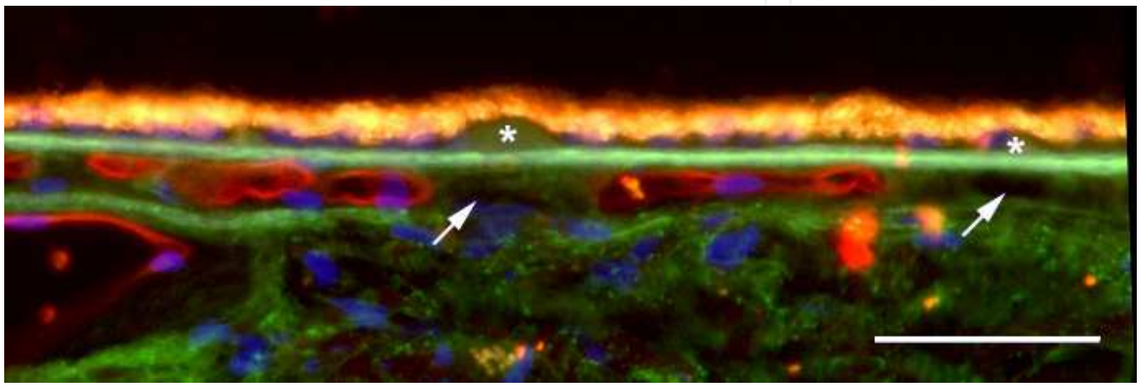

Fig. 5. In human eyes, formation of deposits in the ECM, such as drusen (asterisks) occur preferentially in areas of choroid without capillary lumens, suggesting that the clearance of extracellular debris by the choriocapillaris may be preventative against drusen formation. Arrows, indicate "ghost" capillary vessels. Green immunoreactivity, anti-elastin; red labeling, UEA-I (a vascular marker); orange autofluorescence in the RPE is due to lipofuscin; blue fluorescence is due to a nuclear counterstain. Scale bar = 50μm.

While atrophic changes in AMD may be most easily attributed to excess material deposited in BrM, at some point in the development of neovascular AMD the structure of BrM becomes compromised. That defects in BrM permit angiogenesis into the retina is clear from both genetic diseases in which large cracks appear in the calcified BrM (i.e., pseudoxanthoma elasticum, discussed above) as well as animal models of CNV in which rupture of BrM is sufficient to induce neovascular AMD-like pathology(Ryan, 1980). The molecular changes in early and late AMD that include loss of anti-angiogenic proteins(Bhutto, et al., 2008) and calcification and fragmentation of BrM elastin(Chong, et al., 2005; Spraul and Grossniklaus, 1997) show that breakdown of BrM elastin occurs during the progression of AMD and provides opportunities for neovascular events. These may be especially problematic since, as noted above, fragments of elastin are sufficient to promote the migration of choroidal EC(Skeie and Mullins, 2008).

9. Potential ECM mediated therapeutics

In view of the many roles of the extracellular matrix in macular health and disease, several potential areas exist for ameliorating the pathogenesis of AMD. Current early-phase clinical trials for non-neovascular AMD directed at extracellular matrix dysfunction are limited to

the complement system and summarized in recent reviews. (Yehoshua et al., 2011; Meleth et al., 2011). Ameliorating AMD by modulating other changes that occur in the ECM remains to be explored. This discussion is not intended to be inclusive but to highlight a few areas in which a better understanding of ECM pathobiology of AMD may be helpful.

As discussed above, aberrant elastin metabolism and perhaps signaling are associated with AMD, especially neovascular AMD. Systemic elastin abnormalities occur in AMD, and the local changes in BrM likely at once both remove a barrier to neovascualization and promote growth of new blood vessels from the choroid. A better understanding of the molecules involved in this signaling may provide additional targets of neovascular AMD to accompany anti-VEGF drugs in some cases. This is vital in the cases of neovascular AMD that do not fully respond to anti-VEGF therapy or that develop 'tachyphylaxis' to anti-VEGF drugs.(Gasperini, et al., 2011; Schaal, et al., 2008)

In addition to the pro-angiogenic effects of the elastin components of BrM, other components of BrM are often anti-angiogenic. This has been noted for several molecules including thrombospondin, as discussed above, endostatin(Marneros, et al., 2007; Mori, et al., 2001) a fragment of collagen type XVIII, and fragments of collagen IV(Lima, et al., 2006). Enhancing the expression of these anti-angiogenic molecules through gene delivery or systemic administration shows promise as another tool against neovascular AMD, especially since decreased expression of these inhibitors occurs during pathogenesis(Bhutto, et al., 2008). A caution for these studies is that increases in BrM collagen IV have been linked to thickening of BrM and the development of subRPE deposits, as noted above.

Apart from interfering with signaling events in the aging macula, it may also be possible and necessary to reconstruct BrM in some cases. With the promise of stem cell mediated therapies for AMD and other maculopathies, having a substrate on which transplanted cells can attach and perform their normal physiologic functions is a considerable challenge. Replacing defective RPE cells has been proposed as a mechanism to ameliorate both neovascular(Tezel, et al., 2007) and atrophic(Du, et al., 2011) AMD. Elegant experiments at delivering RPE cells from a variety of potential sources(da Cruz, et al., 2007) indicate that modifying BrM to accept transplanted cells(Gullapalli, et al., 2004) and/or delivering cells on degradable scaffolds(Thomson, et al., 2010) will be necessary for successful transplantation. Advances in combining materials science with cell biology will be essential for the next generation of treatments for AMD.

In summary, the relationship between the photoreceptor cells/RPE and the vascular supply is complex, and the intervening layer of extracellular matrix is especially susceptible to genetic and age-related changes that impair its function. A better understanding of this complex boundary will provide novel opportunities for therapy.

10. Abbreviations

RPE, retinal pigment epithelium; EC, endothelial cell; BrM, Bruch's membrane; ECM, extracellular matrix; AMD, age-related macular degeneration; GA, geographic atrophy; CNV, choroidal neovascularization; OCT, optical coherence tomography; VEGF, vascular endothelial growth factor

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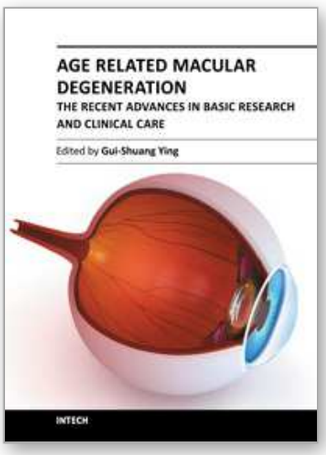

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Age-related Macular Degeneration (AMD) is the leading cause of vision loss and blindness in the developed countries. In the past decade, great progress has been made in understanding the pathobiology and genetics of this blinding disease, as well as in finding new therapies for its treatment. These include the discovery of several genes that are associated with the risk of AMD, new anti-VEGF treatments for wet AMD and new imaging techniques to diagnose and monitor the AMD. All chapters in this book were contributed by outstanding research scientists and clinicians in the area of AMD. I hope this timely book will provide the basic scientists and clinicians with an opportunity to learn about the recent advances in the field of AMD.

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