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Pathogenic Mechanisms

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The outer retina

Age-related changes that predispose to age-related macular degeneration (AMD) occur in the outer retina, more specifically the region that includes the photoreceptors, the retinal pigment epithelium (RPE), Bruch

Retinal anatomy is highly organized and vascular and avascular compartments are strictly segregated in the retina[(1)]. The blood-retinal barriers, inner and outer, are fundamental for the integrity of structure and optimization of function in neuro-sensorial retina[(2)].

The outer blood-retinal barrier is formed, among its various components, by the RPE tight junctions. The intercellular cohesiveness of the RPE is not easily disrupted. Tight junctions appear as a necklace of strands that encircle each cell, binding each cell to its neighbors in a monolayer that separate the outer layer of the neural retina from the choriocapillaris[(3)]. Choriocapillaris is a great vascular network of fenestrated capillaries with high blood flow, fundamental for the metabolism of outer retina. This outer blood-retinal barrier retards transepithelial diffusion through the paracellular spaces[(3)].

The RPE is a polarized epithelium that consist of a continuous pavement-like monolayer of cuboidal shaped cells that in macular area are tall, narrow and highly uniform in size and shape[(4)]. Interdigitation of the apical processes of the RPE with the cone and rod outer segments provides only a tenuous adhesion of the RPE to the sensory retina[(5)].

RPE cells have at least ten known functions, but regeneration of visual pigments, transport of fluids and ions between photoreceptors and choriocapillaris, formation and maintenance of the interphotoreceptor matrix and Bruch's membrane, phagocytosis of outer segments of photoreceptors, and supplying trophic factors such as VEGF-A should be emphasized[(6,7)].

Bruch's membrane is a thin, acellular and well-delineated membrane with five layers. From internal to external these layers are: the basement membrane of the RPE, the inner collagenous zone, the elastic tissue layer, the outer collagenous zone and the basement membrane of the choriocapillaris. It is composed of elements from both, the retina and choroid, but is an integral part of choroid[(8)]. Its inner surface is smooth, whereas its outer surface is composed of a series of collagenous protrusions that extend externally to form the pillars separating and supporting the choriocapillaris[(5)]. Due to its specific location and properties, this tissue is thought to be a vital limiting layer for metabolic transport between the RPE cells and the choriocapillaris[(9)].

The choriocapillaris consists of a continuous layer of fenestrated endothelial cells surrounded by a basement membrane. In the macula the choriocapillaris is arranged in a lobular pattern of highly concentrated interconnecting capillaries supplied by a central arteriole and drained by circumferential venules[(5,10)]. The fenestrations, 60-80 nm in diameter, are abundant and seem to play an important role in permitting the passage of glucose and vitamin A to the RPE and retina. The choriocapillaris supplies oxygen and nutrients to Bruch´s membrane and the outer third of the retina, except in the macula, where it supplies the entire retina[(8)]. The peculiar structure of the choroidal vascular tree in the macula provides this area with the highest rate of blood flow of any tissue in the body[(5)].

Aging changes in outer retina and early AMD

With aging, the neurosensory retina was shown to develop thickening of the internal limiting membrane, diminution of neural elements with age-related loss of rods before cones, gliosis in the peripheral retina, and diminution of capillaries around the fovea, while the lumina of the choriocapillaris and the choroidal thickness become reduced by half[11-13].

With the advancement of age, both the thickness and complexity of Bruch's membrane increase primarily due to extracellular matrix remodeling and accumulation of inclusions in this region[11]. Bruch's membrane calcifies and doubles in thickness between the ages of 10 and 90 years[11]. There is a linear thickening due to deposits of collagen, lipids and debris. After the 30's its lipid concentration increases during life and consequently the fluid permeability and nutrient transport across the membrane decreases[14]. In normal conditions Bruch's membrane acts as an intercellular matrix regulating survival of adjacent RPE and choriocapillaris cells. Its diminished function results in apoptosis of these cells from incorrect cell adhesion[14]. On the other hand extracellular deposits around Bruch's membrane instigate chronic inflammation, invasion by dendritic cells and release of inflammatory cytokines, angiogenic factors and immune complexes[15,16].

The RPE is a monolayer of regularly arranged hexagonal cells that spans the retina from the margins of the optic disc anteriorly to the ora serrata. The number of RPE cells diminishes with age. Macular RPE cells become wither, flatter and increase in height with advancing age[4,17]. In each RPE cell there is a progressive accumulation of lipofuscin during life and in people over 80 years of age, the debris can occupy more than one fifth of the total volume of an RPE cell[18,19]. RPE cells have a brown color in young eyes but with age, they become increasingly more golden colored, owing to the accumulation of lipofuscin pigment granules[20]. Lipofuscin in the RPE is the source of fundus autofluorescence. The major component of lipofuscin is N-retinylidene-N-retinylethanol-amine (A2E), a retinoid product of the visual cycle[21]. The A2E produced interferes with the function of RPE cells, leading to its apoptosis and subsequent geographic atrophy[22]. Age-related changes also include a decrease in the number of melanin granules, loss of basal digitations and irregularity in shape. The RPE cells become separated from their basal membrane by membranous debris and abnormal secretory products and subsequently occurs deposition of collagen and fibronectin and latter formation of basal laminar deposits[23].

Basal laminar deposits are composed of basement membrane protein and long-spacing collagen located between the RPE plasma and basement membranes[24]. Basal laminar deposits are considered the precursors of AMD and can appear around the age of 40 years[25].

Basal linear deposits consist of granular, vesicular or membranous lipid-rich material located external to the basement membrane of the RPE, in the inner collagenous layer of Bruch's membrane, and represent a specific marker of AMD[26].

These two types of deposits can only be shown on pathological specimens and not by clinical evaluation[22].

The combination of the deposits with secondary changes in the RPE results in the formation of drusen. Drusen are localized deposits of extracellular material lying between the basement membrane of the RPE and the inner collagen layer of Bruch's membrane[23,27]. Drusen often have a core of glycoproteins but they also contain fragments of RPE cells, crystallins, apolipoproteins B and E, and proteins related to inflammation such as amyloid P and β, C5 and C5b-9 complement complex[28-31].

Drusen change in size, shape, color, distribution and consistency with the passing years[32].

Small drusen are defined as being less than 63 μm in diameter[33]. The presence of small, hard drusen alone is not sufficient to diagnose early AMD. These deposits are ubiquitous and the new development of small drusen in an adult eye without prior evidence of hard drusen is not age dependent[34].

Hard drusen are discrete nodules or deposits composed of hyaline-like material. During fluorescein angiography hard drusen behave as pin-point window defects[35].

Soft drusen are larger and associated with pigment epithelium detachment and diffuse abnormal Bruch's membrane alterations[36,37]. Soft drusen have a tendency to cluster and merge with one another demonstrating confluence[35]. During fluorescein angiography soft drusen hyperfluoresce early and either fade or stain in the late phase[5].

Drusen can be visible in ophthalmoscopy when their diameter exceeds 25 μm as dots ranging in color from white to yellow[6]. However soft drusen are clinically identified whenever there is sufficient RPE hypopigmentation or atrophy overlying diffuse Bruch's membrane thickening, or, when there are focal detachments within this material. These findings suggest that the clinical identification of soft drusen

identifies an eye with diffuse changes at the RPE-Bruch's membrane complex[35]. When they become larger (>125 µm), and greater the area that they cover, the risk of late AMD becomes higher[38].

The RPE degeneration and nongeographic atrophy of the RPE are characterized by pigment mottling and stippled hypopigmentation with thinning of the neurosensory retina[39]. Histopathology shows mottled areas of RPE hypopigmentation or atrophy overlying diffuse basal linear and basal laminar deposits[36]. Incidence and prevalence rates of RPE depigmentation are age dependent[34].

Focal hyperpigmentation of the RPE, clinically evident as pigment clumping at the level of the outer retina or sub-retinal space, increases the risk of progression to the late phases of the disease[34,36,40].

Aging is known to be associated with increased oxidative damage and the retina is a fertile environment for reactive oxygen species. Apart from the presence of two retinal blood supplies that generate an highly oxygenated environment, the exposure to high levels of cumulative irradiation, high levels of photosensitizers, large amounts of polyunsaturated fatty acids, readily oxidizable lipid, protein and carbohydrate substrates, and the huge proteolytic burden in the RPE contribute to this particular predisposition to oxidative stress[41,42]. This state of accumulation of toxic elements is said to ultimately tip the balance of the ocular immune privilege towards immune activation and inflammation. The end result of an innappropriate activation of diverse immune pathways, including classical and alternative complement pathways, is an immune-mediated retinal damage and/or an impaired immune-mediated retinal maintenance with RPE damage[13].

With all of the above in mind, Zarbin[43] has summarized his review on AMD pathogenesis in five sequential steps as follows: (1) AMD involves aging changes plus additional pathological changes (ie, AMD is not just an aging change); (2) in aging and AMD, oxidative stress causes RPE and, possibly, choriocapillaris injury; (3) in AMD (and perhaps in aging), RPE and, possibly, choriocapillaris injury results in a chronic inflammatory response within the Bruch membrane and the choroid; (4) in AMD, RPE and, possibly, choriocapillaris injury and inflammation lead to formation of an abnormal extracellular matrix, which causes altered diffusion of nutrients to the retina and RPE, possibly precipitating further RPE and retinal damage; and (5) the abnormal extracellular matrix results in altered RPE-choriocapillaris behavior leading ultimately to atrophy of the retina, RPE, and choriocapillaris and/or choroidal new vessel growth. In this sequence of events, patient's susceptibility to AMD would be determined by both the environment and his genetic profile[43].

Late AMD

The primary clinical characteristic of late dry AMD is the appearance of geographic atrophy of RPE. On microscopy, geographic atrophy is seen as abnormal RPE cells with hypotrophy, atrophy, hypertrophy, hypopigmentation, hyperpigmentation, migration, loss of photoreceptors, attenuation of Bruch's membrane and choriocapillaris degeneration[44,45]. Geographic atrophy is clinically characterized by roughly oval areas of hypopigmentation that allows for increased visualization of the underlying choroidal vessels and is the consequence of RPE cell loss. Loss of RPE cells leads to gradual degeneration of photoreceptors and thinning of the retina that may extend to the outer plexiform and inner nuclear layers[6, 29]. Compensatory RPE cell proliferation leads to hyperpigmentary changes frequently observed at the periphery of the hypopigmented areas[6]. The atrophy of RPE is usually more severe than the loss of choriocapillaris but the choriocapillaris seem to be highly constricted in areas of complete RPE cell loss[44].

In neovascular AMD early choroidal neovascularization occurs under the RPE[46]and eventually breaks through[47], leading to accumulation of lipid-rich fluid under the RPE or neuroretina. In haemorrhagic forms blood breaks through the RPE into the subretinal space and sometimes through the retina and into the vitreous[35].

The pattern of growth of CNV often simulates that of a sea fan with radial arterioles and venules supplying and draining a circumferential dilated capillary sinus[5].

As neovascularization of the sub-RPE space occurs, initially the blood flow through the neovascular network is sluggish and there is little or no exudation. This is a period of occult neovascularization and the overlying RPE and neuroretina may be minimally affected[5]. With an increase of blood flow through the network the endothelium decompensates and exudation extends into the subpigment epithelial space creating in some cases RPE detachments. The exudation may also extend through the RPE and detach the overlying retina.

In type II CNV the new vessels extend from the choroid through defects in Bruch´s membrane enters the space between the photoreceptors and RPE cells and growth laterally in the subretinal space[5]. This is usually accompanied by varying amounts of subretinal exudates and/or blood.

Macrophages have been documented both morphologically and functionally in neovascular AMD[48,49]. Activated macrophages and microglia may secret cytokines and chemokines that promote cellular damage and angiogenesis[50].

Involution of CNV eventually occurs and is associated with varying degrees of subretinal scar tissue, reactive hyperplasia of the RPE and/or atrophy, and can partially or totally replace the neuroretina[21]. The outer nuclear layer can be severely attenuated with a reduction of photoreceptor length of almost 70%[51]. Often anastomosis between the retinal circulation and the underlying choroidal circulation develops within these old disciforme scars[32,52].

Other factors like complement factor H, that downregulates the alternative complement pathway[53], HtrA1 – a secretory protein and an inhibitor of transforming growth factor β (TGF-β)[54]– and ARMS2[55] play a role in development of AMD. However its specific role and relevance in development and progression to neovascular and atrophic forms of AMD are discussed in others chapters of this book.

Key points

- Age-related changes that predispose to AMD occur in the outer retina, more specifically the region that includes the photoreceptors, the RPE, Bruch's membrane and the choriocapillaris.
- The deposition of insoluble material, the calcification and increase in thickness of Bruch's membrane, and a less fenestrated and thinner choriocapillaris leads to photoreceptors/RPE hypoxia. The number of RPE cells reduces with age and in each cell there is a progressive accumulation of lipofuscin during life. Basal laminar and basal linear deposits, drusen, RPE degeneration and atrophy develop and finally, geographic atrophy of RPE and choroidal neovascularization occur.
- Choroidal neovascularization is a relative self-limited disease with development, growth and involutional stages leading to the formation of a scar and destruction of macular structure and function.

>> References

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