Molecular Aspects in Choroidal Vasculature

Subjects: Ophthalmology
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Choriocapillaris (CC) density decreases significantly with aging and may have a systemic basis. Reduced perfusion of the CC, in combination with changes in the thickness and composition of Bruch's membrane (BrM), further aggravates the metabolic homeostasis of the retinal pigment epithelium (RPE) and thus the photoreceptor cells. Indeed, the age-related modifications of the choroidal vasculature have been reported to be associated with a wide range of BrM alterations, basal linear and basal laminar deposits, and RPE changes. The arterial supply of the choroid is segmental from the PCAs to the CC. The segmental and lobular organization of choroidal vasculature can be appreciated at both the posterior pole and the equatorial retina.

choroid retina age-related macular degeneration choroidal vascularity index

1. Genetic Factors Associated with Pathologic Choroid in AMD

Complement factor (*CFH)* rs1061170 risk genotype, a well-known single nucleotide polymorphism (SNP) in AMD, was associated with choroidal thinning (−14.7% with the risk allele C) in healthy elderly individuals, suggesting a common pathway involving choroidal thinning in AMD pathogenesis [1]. Similar results were reported by Mullins et al., who revealed that the eyes homozygous for the Y402H allele presented significant choroidal thinning, compared with those homozygous for the low-risk Y allele (−23.6% thinner). The authors hypothesized that the *CFH* polymorphisms associated with impaired protein function or localization could be associated with the increased formation of membrane attack complex (MAC) and further complement activation, leading to CC injury in early AMD [2].

Most studies investigating the association between genetic polymorphisms and choroidal vascular changes involved a specific subtype of neovascular AMD (nAMD), namely polypoidal choroidal vasculopathy (PCV). This phenotype represents almost 50% of nAMD cases in the Asian population and is characterized by nodular or aneurysmatic dilatations arising from the neovascular network [3]. The I62V polymorphism in the *CFH* gene contributed to subfoveal choroidal thickening in eyes with PCV, while the Y402H polymorphism in the *CFH* gene and the A69S polymorphism in the age-related maculopathy susceptibility2 (*ARMS2*) gene were not associated with choroidal thickness. Since the *CFH* gene is a key regulator of the alternative pathway of the complement system, the authors speculated a possible link between inflammation and choroidal modifications in PCV eyes [4]. Another study on eyes with PCV reporting the factors associated with subfoveal choroidal thickening included

younger age, shorter axial length, G-allele frequency in *ARMS2* A69S (rs10490924), and T-allele frequency in *CFH* (rs1329428) [5].

2. Molecular Properties of the Choroidal Vascular Layer

Human leukocyte antigen (HLA) expression has been demonstrated in both healthy and AMD eyes and is mainly expressed on the endothelial cells of the CC. An increased HLA class II immunoreactivity was also observed in AMD eyes during drusen formation [6][7]. The association of HLA antigens in AMD suggests a pathogenetic role in driving the cellular immune-mediated response against local antigens or newly presenting antigens, especially drusen and AMD-related evolutive changes [6].

As mentioned above, ICAM-1 is constitutively expressed in the endothelial cells of the CC in healthy subjects. Its expression is associated with leukocyte recruitment in the choroid, thus expanding the immune response [8][9].

The endothelial cells of the CC are particularly dependent upon VEGF, which is crucial for CC development and maintenance [10][11][12][13]. VEGF secretion is also essential in maintaining the endothelial CC fenestrations, and VEGFR-2 is actively involved in CC development and maturation during gestational age [14].

Mast cells play crucial roles in inflammation and are involved in several pathophysiological processes, including wound healing, tissue regeneration and remodeling after injury, and angiogenesis [15]. Mast cells and macrophages are the main leukocytes detectable in the choroid and proved to be involved in the pathogenesis of AMD [16].

The complement system is a molecular cascade part of the innate immune system that drives the inflammatory response, cooperates with antibodies to destroy pathogens, and improves the individual immune response. When activated, the complement cascade proteases cleave specific proteins, releasing the cytokines that further activate and amplify the cascade. These events initiate the cell-killing membrane attack complex (MAC) that perforates cellular membranes. The complement system consists of three distinctive pathways: the classical, the alternative, and the mannose-binding lectin pathway. The classical pathway is triggered by the antigen–antibody complexes that bind the C1 complex. The alternative and mannose-binding lectin pathways are activated without needing any antigen–antibody interaction. The complement system is constantly activated at low levels in normal eyes under the control of the complement regulatory proteins that maintain a balanced activation to avoid eye damage [17].

3. Age-Related and Pathologic Changes

The interaction between leukocytes and BrM is mainly related to the topographical distribution of drusen and basal deposits. Collagenous debris is distributed within and beneath BrM, representing a target of phagocytic activity. In the early stages of a disease, the cellular interactions are limited to the BrM, whereas at later stages, the cells can be visible on the inner retinal surface through breaks of the BrM. These breaks may favor the passage of neovessels with a possible role in the choroidal neovascularization progression [18].

Mast cells are resident choroidal inflammatory cells that tend to increase in all the choroidal areas of eyes with early AMD. Mast cells appeared degranulated and located close to the CC or in spaces where the CC had degenerated. An increasing number of degranulated mast cells were also evident in the paramacular choroidal locations of eyes with exudative AMD and GA. The degranulated mast cells in neovascular AMD were also located adjacent to the feeder vessels passing through BrM breaks, in the basal laminar deposits overlying neovascularization, or within lesions. Moreover, the mast cells were primarily located in the Sattler's layer and CC areas with complete RPE atrophy in GA eyes [15]. In eyes with high-risk genetic loci on chromosomes 1 and 10, increased mast cell proteases and choroidal mast cells were evident in the absence of clinical AMD changes and after AMD had developed. These findings suggested that mast cell infiltration and degranulation are early events in AMD pathogenesis. Furthermore, the mast-cell-protease-mediated degradation of the CC basement membrane leads to endothelial damage. However, the altered integrity of BrM also facilitates the access of large blood-derived proteins between the RPE–basal lamina and the inner collagenous layers, causing drusen formation and progressive RPE damage [19].

Resident choroidal macrophages are absent in normal human eyes without inducible nitric oxide synthase (iNOS) expression. In eyes with early AMD (group III-IV) with pigmentary changes, macrophages occupied choroidal capillaries or the intercapillary pillars. In this context, the choroidal vascular endothelial cells and perivascular cells also expressed iNOS. In the same group, the macrophages were initially located in correspondence with soft drusen, but they tended to increase in the presence of basal laminar deposits, being recognizable in all the examined eyes as the deposits became thicker. When choroidal neovascularization occurred, the macrophages further increased in number and frequently eroded the intercapillary pillars of BrM. In the context of GA, the macrophages were reduced underneath the atrophic area but were mainly distributed at the atrophic border and adjacent to viable RPE. In disciform scarring, the macrophages were detectable within the inactive and active scar, while iNOS expression was only appreciated within the active lesions [20].

The dysregulation of complement activity with the evidence of C3 and C5 complement fragments and MAC C5b-9 have been identified in the context of drusen and choroidal capillary pillars, as well as in the vitreous of AMD eyes [17][21][22]. The common genetic polymorphisms carrying an increased risk for AMD involve the regulation of the complement pathway. A polymorphism (Tyr402His) in the CFH gene, which mainly controls the alternative complement activation, reduces the affinity for C-reactive protein (CRP), increasing the levels of unbound CRP in the choroid, leading to uncontrolled chronic inflammation [22][23][24].

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