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Pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) in aged human choroid and eyes with age-related macular degeneration

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Abstract

The purpose of this study was to examine the localization and relative levels of vascular endothelial growth factor (VEGF; an angiogenic factor) and pigment epithelium-derived factor (PEDF; an antiangiogenic factor) in aged human choroid and to determine if the localization or their relative levels changed in age-related macular degeneration (AMD).

Ocular tissues were obtained from eight aged control donors (age range, 75–86 years; mean age, 79.8 years) with no evidence or history of chorioretinal disease and from 12 donors diagnosed with AMD (age range, 61–105 years; mean age, 83.9 years). Tissues were cryopreserved and streptavidin alkaline phosphatase immunohistochemistry was performed with rabbit polyclonal anti-human VEGF and rabbit polyclonal anti-human PEDF antibodies. Binding of the antibodies was blocked by preincubation of the antibody with an excess of recombinant human PEDF or VEGF peptide. Choroidal blood vessels were identified with mouse anti-human CD-34 antibody in adjacent tissue sections. Three independent observers graded the immunohistochemical reaction product.

The most prominent sites of VEGF and PEDF localization in aged control choroid were RPE–Bruch's membrane–choriocapillaris complex including RPE basal lamina, intercapillary septa, and choroidal stroma. There was no significant difference in immunostaining intensity and localization of VEGF and PEDF in aged control choroids. The most intense VEGF immunoreactivity was observed in leukocytes within blood vessels. AMD choroid had a similar pattern and intensity of VEGF immunostaining to that observed in aged controls. However, PEDF immunoreactivity was significantly lower in RPE cells ($p=0.0073$), RPE basal lamina ($p=0.0141$), Bruch's membrane ($p<0.0001$), and choroidal stroma ($p=0.0161$) of AMD choroids. The most intense PEDF immunoreactivity was observed in disciform scars. Drusen and basal laminar deposits (BLDs) were positive for VEGF and PEDF.

In aged control subjects, VEGF and PEDF immunostaining was the most intense in RPE-Bruch's membrane-choriocapillaris complex. In AMD, PEDF was significantly lower in RPE cells, RPE basal lamina, Bruch's membrane and choroidal stroma. These data suggest that a critical balance exists between PEDF and VEGF, and PEDF may counteract the angiogenic potential of VEGF. The decrease in PEDF may disrupt the balance and be permissive for the formation of choroidal neovascularization (CNV) in AMD.

Keywords: choroid; pigment epithelium-derived factor; vascular endothelial growth factor; RPE; Bruch's membrane; age-related macular degeneration; choroidal neovascularization

1. Introduction

Age-related macular degeneration (AMD) is one of the leading causes of irreversible visual loss among people 65 years of age and older in the western world (Ambati et al., 2003). The pathogenesis of AMD is probably multifactorial; its late onset, complex genetics, and strong environmental components may all contribute at some

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level. Choroidal neovascularization (CNV), growth of new blood vessels from the choroid, occurs in the advanced 'exudative' form of AMD and is the major risk factor for blindness. Exudative AMD is characterized by submacular ingrowth of choroidal vessels through a damaged Bruch's membrane that may remain beneath the retinal pigment epithelium (RPE) or breach the RPE layer and enter the subretinal space. The pathologic vasculature leaks serous fluid and/or blood and ultimately causes a disciform scar in and under the macular region of retina (Green and Key, 1977; Green et al., 1985). Unlike retinal neovascularization, CNV in AMD is not an obviously ischemia-driven disease. In fact, no unifying theory currently exists for the growth of abnormal blood vessels in the subretinal space in AMD and other related diseases. Ascertaining what initiates the growth of CNV in AMD is one of the main challenges in this field of research.

There is evidence to suggest that the net balance between endogenous angiogenic and anti-angiogenic growth factors exists in normal eye and an imbalance of these factors induces growth of new blood vessels inwards from the choroid. The presence of angiogenic growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2) in surgically removed choroidal neovascular membranes and in experimental-induced CNV in animal models has been demonstrated (Amin et al., 1994; Kvanta et al., 1996; Ishibashi et al., 1997). These previous studies also suggest that abnormalities of the extracellular matrix of RPE cells may promote a proangiogenic phenotype and the development of CNV.

In addition to proangiogenic factors, it has recently become apparent that a variety of endogenous antiangiogenic factors like pigment epithelium-derived factor (PEDF), endostatin, and thrombospondin (TSP) may contribute to vascular quiescence. PEDF, one of the endogenous antiangiogenic factors found in the eye, was first purified from the conditioned medium of human RPE cells (Tombran-Tink et al., 1991). It is a member of the serine protease inhibitor (serpin) family with neuroprotective, neurotrophic (Tombran-Tink et al., 1991), and antiangiogenic activities (Dawson et al., 1999). It's potential as a therapeutic agent for retinal and choroidal diseases triggered by photoreceptor degenerations and abnormal neovascularization has been explored in animal models (Mori et al., 2001, 2002a; Gehlbach et al., 2003) and is being evaluated in a clinical trial (Rasmussen et al., 2001). Although in vivo expression of PEDF in human choroid with AMD still remains to be elucidated, PEDF has been demonstrated in choroid of animal models and suggested to play an important role in experimental choroidal neovascular membrane formation (Ogata et al., 2002). The counterbalance of VEGF and PEDF is supported by the previous demonstrations that either inhibition of the VEGF system or over expression of PEDF inhibits choroidal neovascularization (Krzystolik et al., 2002; Mori et al., 2002b).

The goal of the current study was to determine the localization and relative levels of VEGF and PEDF in aged human choroid and RPE and to evaluate the changes in PEDF and VEGF localization and intensity in AMD. The data suggest that the levels of these two important endogenous proteins may regulate CNV.

2. Materials and methods

2.1. Donor eyes

Human donor eyes were obtained with the help of Janet Sunness, M.D. and Carol Applegate at the Wilmer Ophthalmological Institute (Baltimore, MD) and the National Disease Research Interchange (NDRI; Philadelphia, PA). Eyes of the following donors were used in the study: 12 subjects with AMD (age range, 61–105 years; mean age, 83.9 ± 13.1 years); eight aged control donors (age range, 75–86 years; mean age, 79.8 ± 3.7 years) with no medical history of chorioretinal disease. All donors were Caucasian. Table 1 includes the postmortem time (PMT) and death-to-enucleation time (DET), the age, sex, cause of death, and the medical and ocular history for each subject. The protocol of the study adhered to the tenets of the Declaration for Helsinki regarding research involving human tissue. The diagnosis of AMD was made by reviewing ocular medical history on the eye bank transmittal sheet and the postmortem gross examination of the eyecup using transmitted and reflected illumination with a dissecting microscope (Stemi, 2000; Carl Zeiss, Inc., Thornwood, NY).

2.2. Tissue preparation and sectioning

After a circumferential incision was made 0.5 cm posterior to the limbus, the anterior segment of the eye was removed, and the eyecup was examined by stereomicroscopy (Stemi 2000; Carl Zeiss, Inc., Thornwood, NY). Gross images were obtained with a digital camera (QImaging; Vancouver, BC, Canada) and imported directly into image analysis software (Photoshop ver. 6.0; Adobe Systems Inc., San Jose, CA, on a PowerMac G3; Apple Computer, Cupertino, CA). Eyes were fixed in 2% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) at room temperature for 1 hr, cryopreserved with increasing concentrations of sucrose, and 8 μM sections were cut from the macula as previously described (Lutty et al., 1993).

2.3. Immunohistochemistry

Streptavidin alkaline phosphatase (APase) immunohistochemistry was performed on cryopreserved tissue sections using a nitroblue tetrazolium (NBT) development system as previously described (Bhutto et al., 2004). In brief, 8 μM