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PHOTORECEPTOR DEGENERATION, STRUCTURAL REMODELING AND GLIAL ACTIVATION: A MORPHOLOGICAL STUDY ON A GENETIC MOUSE MODEL FOR PERICYTE DEFICIENCY

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Abstract—Interaction between pericytes and endothelial cells via platelet-derived growth factor B (PDGF-B) signaling is critical for the development of the retinal microvasculature. The PDGF-B retention motif controls the spatial distribution range of the growth factor in the vicinity of its producing endothelial cells allowing its recognition by PDGF receptor beta-(PDGFR-β)-carrying pericytes; this promotes recruitment of pericytes to the vascular basement membrane. Impairment of the PDGF-B signaling mechanism causes development of vascular abnormalities, and in the retina this consequently leads to defects in the neurological circuitry. The vascular pathology in the *pdgf-b*$^{ret/ret}$ (PDGF-B retention motif knockout) mouse retina has been previously reported; our study investigates the progressive neuronal defects and changes in the retinal morphology of this pericyte-deficient mouse model. Immunohistochemical analysis revealed retinal injuries to occur as early as postnatal day (P) 10 with substantial damage progressing from P15 and onward. Vascular abnormalities were apparent from P10, however, prominent neuronal defects were mostly observed from P15, beginning with the compromised integrity of the laminated retinal structure characterized by the presence of rosettes and focally distorted regions. Photoreceptor degeneration was observed by loss of both rod and cone cells, including the disassembly and altered structure of their synaptic terminals. Significant shortening of cone outer segments was observed from P10 and later stages; however, decrease in cone density was only observed at P28. Disorganization and dendrite remodeling of rod bipolar cells also added to the diminished neural and synaptic integrity. Moreover, in response to retinal injuries, Müller and microglial cells were observed to be in the reactive phenotype from P15 and onward. Such a sequence of events indicates that the *pdgf-b*$^{ret/ret}$ mouse model displays a short time frame between P10 and P15, during which the retina shifts to a retinopathic phase by the development of prominently altered morphological features.

Key words: photoreceptor degeneration, cell death, rosette, synapse, microglia, Müller cells.

INTRODUCTION

The interaction between pericytes and endothelial cells is imperative in the development of a functional vascular system. In the retinal microvasculature, where pericyte coverage is highest among other organs, these key cells not only participate in vascular development, stabilization, maturation, and remodeling, but also in the formation of the blood–retinal–barrier (Armulik et al., 2011; Schallek et al., 2013). During developmental angiogenesis, platelet-derived growth factor receptor beta (PDGFR-β)-expressing pericytes are recruited to the vascular basement membrane by platelet-derived growth factor B (PDGF-B)-secreting endothelial cells. Further proliferation and migration of the pericytes along the blood vessel walls occur through this constant paracrine signaling with the endothelium (Lindahl et al., 1997; Benjamin et al., 1998; Hellström et al., 1999, 2001). Genetic manipulation of PDGF-B and its receptor is found to result in pericyte deficiency with phenotypes depictive of vascular dysfunction. Severe vascular defects are evident in PDGF-B-and PDGFR-β-null mice where abnormalities related to vessel dilation, tortuosity, microaneurysms, endothelial cell hyperplasia and vascular rupture cause severe hemorrhaging and compromised cardiovascular function with consequent embryonic lethality (Levéen et al., 1994; Soriano, 1994; Lindahl et al., 1997; Hellström et al., 1999, 2001).

The proper integration of pericytes to the vasculature is also dependent on the presence of a basic sequence of amino acids known as the 'retention motif' located at the carboxy terminal of PDGF-B. The retention motif allows PDGF-B to bind to heparan sulfate proteoglycans as well as other extracellular matrix molecules and thereby controls the spatial distribution limit of the

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Abbreviations: α-sma, alpha smooth muscle actin; BSA, bovine serum albumin; cc-3, cleaved caspase-3; Cho, choroid; GFAP, glial fibrillary acidic protein; IB4, isolectin B4; Iba1, ionized calcium binding adapter molecule 1; INL, inner nuclear layer; NG2, neuron-glial antigen 2; ON, optic nerve; ONL, outer nuclear layer; OPL, outer plexiform layer; P, postnatal day; PBS, Phosphate-buffered saline; PDGF-B, Platelet-derived growth factor B; PDGFR-β, Platelet-derived growth factor receptor beta; *pdgf-b*$^{ret/ret}$, platelet-derived growth factor b retention motif knockout; PKCα, protein kinase C alpha; PNA, peanut agglutinin; Rho, rhodopsin; RPE, retinal pigment epithelium; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; TSPO, translocator protein.

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growth factor in close proximity to the producing endothelial cells. The localization of secreted PDGF-B in the vicinity of the endothelium allows for its recognition by the PDGFR-β-carrying pericytes; moreover, depots of localized PDGF-B create gradients that stimulate pericyte migration along the microvessel abluminal surface among restricted paths (Abramsson et al., 2003; Lindblom et al., 2003; Armulik et al., 2011). The PDGF-B retention motif knockout (*pdgf-b*$^{ret/ret}$) mouse not only shows pericyte deficiency, with a pericyte density reduction of ~50%, but also partial detachment of pericytes from the vascular walls. The combined effect of these factors leads to severe vascular dysfunction similar to, but somewhat milder than, *pdgf-b*$^{-/-}$ and *pdgfr-β*$^{-/-}$ mice; however, unlike the PDGF-B and PDGFR-β null mutants these mice are postnatally viable (Abramsson et al., 2003; Lindblom et al., 2003). As the retinal vessels develop during the first postnatal week (Fruttiger, 2007), early changes in vascular permeability and the onset of retinopathy can be analyzed in the *pdgf-b*$^{ret/ret}$ mouse. This model starts showing retinal vessel abnormalities as early as postnatal day (P2) with severe retinal morphology at P180 (Lindblom et al., 2003). Other PDGF knockout models demonstrate retinopathy after the third postnatal week (Enge et al., 2002).

From extensive studies on detached retinas, it has been documented that the retinal cells display a considerable repertoire of cellular responses to various injuries (Fisher et al., 2005). Photoreceptor apoptosis leads to sensory deafferentation of the inner retina, and is usually followed by remodeling of postsynaptic bipolar and horizontal cells. Other secondary responses of the neurosensory retina are activation and remodeling of the Müller cells and resident microglia. Müller cell activation, in particular, leads eventually to increased tractional forces in the tissue and a functionally deteriorated retina with altered morphology (Lewis and Fisher, 2003; Jones and Marc, 2005). Glia cell reactivity is also associated with increases of pro-inflammatory mediators, which in turn have been suggested to trigger photoreceptor degeneration (Tang and Kern, 2011). Subtle responses in photoreceptor axon terminals of the affected retina in experimental models may include impaired synapse function, retraction, and/or sprouting of axon terminals into the inner retina (Fuchs et al., 2012; Linberg et al., 2009; Specht et al., 2007; VanGuilder et al., 2008).

The retinal microvasculature is intimately related to the survival and functional capacity of the neurological circuit. Severe vascular dysfunction has been related to vision loss associated with age-related macular degeneration, proliferative diabetic retinopathy, retinopathy of prematurity and glaucoma (Caprara and Grimm, 2012). The vessel abnormalities associated with pericyte loss and detachment in the *pdgf-b*$^{ret/ret}$ mouse retina have been previously reported (Lindblom et al., 2003); here we focus our study on the neurological defects that may lead to retinopathy and possible vision impairment as a result of genetic knockout of the PDGF-B retention motif.

The major aim of this study was to characterize the *pdgf-b*$^{ret/ret}$ mutant mouse retina with emphasis on neurodegenerative events related to photoreceptor death, cone photoreceptor plasticity, synaptic disassembly, neural remodeling and gliotic changes during the first four postnatal weeks. We found that several signs of neural remodeling and gliotic changes developed in the *pdgf-b*$^{ret/ret}$ mouse retina after the first postnatal week. Most prominent features were the appearance of rosette formations, photoreceptor cell death, underdeveloped cone outer segments, disruption of synaptic integrity between photoreceptors and interneurons as well as Müller cell and microglia reactivity. Our findings demonstrate that the *pdgf-b*$^{ret/ret}$ mouse shows several structural alterations in the retinal architecture in response to vascular malfunction and neural defects.

EXPERIMENTAL PROCEDURES

Animals

Retinas were studied in transgenic mice lacking the PDGF-B retention motif (*pdgf-b*$^{ret/ret}$), backcrossed at least seven generations against C57Bl/6-J and identified by PCR genotyping as described earlier (Nisancioglu et al., 2010). All procedures were carried out in accordance with institutional policies following approval from the Animal Ethics Board of Northern Stockholm.

Control and *pdgf-b*$^{ret/ret}$ mice were decapitated followed by prompt enucleation of the eyes at P 7, 10, 15 and 28. Small incisions in the cornea were made prior to immersion in fixative consisting of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) (pH 7. 4), for 8–24 h at 4 °C. After cryoprotection in PBS containing increasing sucrose concentrations (10–30%) at 4 °C, the retina and optic nerve (ON) were separated from the cornea, lens and vitreous by a circumlinear incision under a dissection microscope. The retinal specimens were embedded in OCT medium and sectioned at 10–12-μm thickness with a cryostat.

Immunohistochemistry

Rod photoreceptor cells were identified using a mouse monoclonal antibody against rhodopsin (Rho) (1:2000; Millipore, Bedford, MA, USA), and apoptotic photoreceptors were identified using a rabbit anti-cleaved caspase-3 (cc-3, 1:1000; Cell Signaling Technology, Beverly, CA, USA). A rabbit polyclonal antibody against ribeye (1:1000; Synaptic Systems GmbH, Göttingen, Germany) was used to label ribbon protein in the photoreceptor synaptic terminals. A rabbit polyclonal antibody against protein kinase C alpha (PKCα, 1:1000; Santa Cruz) was used to identify rod bipolar cells. Activated Müller cells were identified using a rabbit polyclonal antibody (1:2000; ProteinTech Group, Chicago, IL, USA) against glial fibrillary acidic protein (GFAP). Ramified and reactive macrophage/microglial cells were labeled with a rabbit polyclonal antibody against ionized calcium binding adapter molecule 1 (Iba1, 1:2000; ProteinTech) and rabbit monoclonal antibody against translocator protein (TSPO, 1:200; Abcam, Cambridge, UK). Pericytes were