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Retinal Ultrastructural and Microvascular Defects in Decorin Deficient (*Dcn*$^{-/-}$) Mice

Rayne R. Lim[1,2], Suneel Gupta[1,3], DeAna G. Grant[4], Prashant R. Sinha[1,3], Rajiv R. Mohan[1,2,3], Shyam S. Chaurasia[1,2]

[1.] Ocular Immunology and Angiogenesis Lab, Department of Veterinary Medicine & Surgery, University of Missouri, Columbia, MO 65211, USA
[2.] Department of Biomedical Sciences, University of Missouri, Columbia, MO 65211, USA
[3.] Harry S. Truman Memorial Veteran Hospital, Columbia, MO 65211, USA
[4.] Electron Microscopy Core, University of Missouri, Columbia, MO 65211, USA

Retinal angiogenic diseases (RADs) are the leading cause of visual impairment in working adults. RADs included conditions such as age-related macular degeneration, diabetic macular edema, and diabetic retinopathy. These diseases are asymptomatic in the early stages, contributing to the difficulty in diagnosis and treatment. Vascular abnormalities including microaneurysms, dot hemorrhages and neovascularization are central themes of RADs [1]. Therefore, therapies such as intravitreal injection of anti-VEGF drugs and laser photocoagulation surgery are targeted to halt disease progression only in the advanced stages of the diseases. However, approximately 50% of patients do not respond, and often require multiple treatments. There is no current treatment for the early stage of RADs. Hence there is an urgent need to understand the components involved in vascular angiogenesis, specifically the extracellular matrix (ECM) which contributes to the integrity of the microvasculature in the retina.

Decorin (Dcn) is a small leucine-rich proteoglycan (SLRP) with multiple functions. It is involved in fibrillogenesis and regulation of ECM mechanical properties by binding and interacting with their major components. Dcn is also involved in modulating inflammation as it binds to Toll-like receptor 4 (TLR4) and sequesters proinflammatory cytokine – TNFα and growth factors such as transforming growth factor beta (TGFβ) [2,3]. Furthermore, evidence shows that Dcn abrogates angiogenesis by direct inhibition of vascular endothelial receptor 2 (VEGFR2) [4]. In the retina, Dcn plays a key role as a neurotrophic factor during retinal differentiation [5], and localizes to nerve fiber layer and ganglion cell layer in the mature retina [6]. Upon retinal injury in mice, Dcn was shown to be highly upregulated in the inner retinal layers [7], while treatment with decorin was shown to improve vitrectomy outcome in rabbits with proliferative vitreoretinopathy [8], and prevent loss of tight junctions in human retinal pigmented epithelial cells under hyperglycemic and hypoxia stress [9]. Taken together, results indicate Dcn to be actively involved in retinal repair. However, its function in the maintenance of adult retinal microvasculature is not known. Hence this study uses Dcn knockout (*Dcn*$^{-/-}$) mice to elucidate the functional role of Dcn in preserving the neuronal structural integrity and spatial organization in a retinal microvasculature.

Eight to twelve weeks old *Dcn*$^{-/-}$ mice retina were photographed using fundus photography and fundus fluorescein angiography. Animals were sacrificed and eyes enucleated for analysis. Eyes were fixed in paraformaldehyde (PFA), dehydrated and embedded in paraffin for histological examination. Retina fixed in formalin were isolated from eyecups and subjected to trypsin digestion to visualize the intact retinal vasculature. Retinal flat-mount staining with isolectin GS-IB4 was performed to examine capillary integrity. Electron microscopy was used for ultrastructural investigation of retinal and neuronal cells. For EM preparation, enucleated mouse eyes were dissected immediately in the primary fixative

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medium of 2% PFA and 2% glutaraldehyde in 100 mM sodium cacodylate buffer. The anterior tissues including cornea, ciliary body and lens were removed. Entire remaining eye cup was immobilized in histogel, and secondary fixed using 1% osmium tetroxide in cacodylate buffer. En bloc staining was performed using 1% aqueous uranyl acetate. After overnight incubation in 4°C, ethanol was used for graded dehydrated (100 Watts for 40s, per exchange), before transitioning into acetone and infiltration with Epon resin (250 Watt for 3 min). Polymerization was done at 60°C overnight. Retinal eyecups were halved transversely to reach the center of the retina near the optic nerve. Tissue block was trimmed to 500µm length and cut with a diamond knife to yield 75nm sections for TEM. Images were acquired with a JEOL JEM 1400 transmission electron microscope at 80 kV on a Gatan Ultrascan 1000 CCD, at 1200X magnification.

Retinal imaging revealed *Dcn*$^{-/-}$ mice to exhibit irregular vasculature with reduced caliber (thickness), increased tortuosity (curvature) and fractal dimensions (hyper-branching). This was supported by the flat-mount isolectin GS-IB4 staining, which also showed the increased migration of stained microglial cells to the inner layers of the retina, indicative of retinal damage and stress. Trypsin digest isolated retinal vasculature displayed capillaries with irregular thickness, flattened endothelial cells, and abnormal pericyte morphology in *Dcn*$^{-/-}$ mice. Increased number of acellular capillaries were also observed, indicative of vascular damage in these knockout mice. Surprisingly, cross-section examination of the retina with histology staining showed severe thinning in the *Dcn*$^{-/-}$ mice, with loss of thickness across all layers of the retina. Electron microscopy analysis confirmed photoreceptor degeneration, along with the loss of neuronal cells across all the layers of retina similar to pathological conditions seen in RADs.

This is the first study defining the role of Dcn in the maintenance of retinal structural integrity and microvasculature. *Dcn*$^{-/-}$ mice exhibit signs of retinal angiogenic disease including neuronal and vascular defects, indicating key roles for Dcn in protection and homeostasis in the retina [10].

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