Introduction

Diabetic retinopathy is the leading cause of blindness among the working age population and a 50% increase in its prevalence is expected by 2030. Pericyte loss, vascular hyperpermeability, and increased vascular endothelial growth factor-A (VEGF-A) production are critical to its pathogenesis. Angiopoietin-1 (Ang1), secreted by pericytes, maintains a stable and hyperpermeability and promoting vascular endothelial (VE)-cadherin stabilization.

Methods

Mice. Mice heterozygous for the Ins2 mutation (C57BL/6-Ins2Akita/J) were used. The age-matched controls (C57BL/6J) were housed in the same environment as the Ins2Akita mice.

AAV2. Constitutive expression of COMP-Ang1 (or control) was achieved with adeno-associated viral vector serotype 2 (AAV2). COMP-Ang1 and AAV2.AcGFP were injected into the vitreous of C57BL/6J and Ins2Akita mice to determine the effects on retinal vasopermeability.

Figure 1

AAV2.COMP-Ang1 retards diabetic retinal capillary dropout (a) Retinal en face images of the retna stained for id3 (endothelial cell marker, red), and iSM (leukocyte marker, green). Ins2Akita mice experienced endothelial and pericyte dropout compared to C57BL/6J mice. Endothelial cell loss was prevented by AAV2.COMP-Ang1.

Figure 2

(a) COMP-Ang1 increases vascular barrier function in human retinal microvascular endothelial cells. (b) COMP-Ang1 increases vascular permeability in diabetic mice. (c) COMP-Ang1 increases VE-cadherin in the mouse retina.

Figure 3

AAV2.COMP-Ang1 prevents diabetes-induced retinal ganglion cell layer degeneration. (a) Cross-sections of retinas stained for GAPDH (grey), anti-Tie2 (red) or nuclei (blue). Right, magnified view of the ganglion cell layer from AKI2-20005 Ang1 and AAV2.AcGFP treated mice demonstrating increased VE-cadherin staining. (b) Optical coherence tomography (OCT) measuring retinal thickness. AAV2.COMP-Ang1 prevented diabetic retinopathy-induced retinal thinning, n = 3 mice/group. *P < 0.01 vs. C57BL/6J. **P < 0.01 vs. AAV2.AC GFP.

Figure 4

(a) COMP-Ang1 preserves visual acuity in diabetic mice. (b) Normalized decrease in b-wave amplitude as a function of stimulus intensity. AAV2.COMP-Ang1 prevented diabetes-induced decrease in visual acuity (P < 0.01 vs. AAV2.AC GFP).

Discussion

The Ins2Akita mouse exhibits several hallmarks of proliferative diabetic retinopathy including increased VEGF-A protein expression, vascular hyperpermeability and hyperpermeability.

Current therapy for diabetic retinopathy focuses on suppressing VEGF either through ablating large portions of the retina with laser photocoagulation or administering anti-VEGF antibodies, neither of which addresses the underlying cause.

We hypothesized that stabilizing the vasculature would promote proper perfusion and prevent the retinal ischemia responsible for increased VEGF production.

AAV2.COMPAng1 prevents diabetes-induced retinal vasopermeability (despite persistent pericyte dropout) retaining thinning and ganglion cell loss visual acuity and retinal function defects.

We propose that AA V2.COMPAng1 normalizes the vasculature by increasing VE-cadherin stability and preventing the endothelial cell lost seen in Ins2Akita mice. The normalized vasculature results in enhanced perfusion, reduced hypoxia-driven VEGF production (further contributing to vascular stability) and reduced ganglion cell layer loss. This in turn prevents decreases in visual acuity and ERG responsiveness, which decrease in diabetic patients due to ischemia.

References


Structure

Retinal vasopermeability. Vascular hyperpermeability was established with Evans Blue method.

Visual Acuity. Optomotor head tracking response was used to determine visual acuity.

Retinal electrofunction. Electroretinography (ERG) was performed on anesthetized mice under scotopic conditions.