Knockdown of Müller cell specific VEGF reduces retinal neovascularization in a rat model of retinopathy of prematurity

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Purpose

To determine the effects from knockdown of Müller cell expressed vascular endothelial growth factor (VEGF) in the 50/10 model of oxygen induced retinopathy (50/10 OIR model).

Introduction

Retinopathy of prematurity (ROP) remains a leading cause of childhood blindness and is increasing in frequency in developing countries. The pathophysiology of ROP can be described by two stages: 1) first, delay in physiological vascular development causing avascular retina and 2) later abnormal neovascularization that grows into the vitreous.

VEGF is one of the most studied factors leading to IVNV. VEGFA protein was found increased in vitreous from preterm infants who had surgery for stage 4 ROP compared to controls. Following a recent clinical trial testing intravitreal delivery of a broad anti-VEGF antibody in infants with severe ROP, there have been concerning reports of persistent avascular (AVA) retina and new intravitreal neovascularization (IVNV) with total retinal detachment, even one year after surgery. Using a relevant model of ROP, we found that inhibition of VEGF with a neutralizing intravitreal antibody not only led to recurrent IVNV and reduced retinal neovascular growth, but also upregulation of angiogenic factors, including angiopoietin-2. Targeting the cell type that overexpresses VEGF may lead to safer treatments for ROP.

Methods

1. Lentivirus with shRNA to VEGF targeting Müller cells: As previously described, two shRNAs to VEGFA or luciferase were created and tested for specificity to VEGF knockdown using reporter cell lines in HEK293 cells. The shRNA sequence for the greater knockdown was then encoded within a miR30 microRNA and cloned into the lentivector pFMCD44 with a GFP tag that was shown to target Müller cells. Sensitivity and specificity of the lentivector was confirmed in vitro and in vivo and was presented previously.

2. Animal model: As reported, within 4 hours of birth, pups and their dams were placed into an Oxycycler (Biox Oxygen, NY), which cycled oxygen between 50% and 10% every 24 hours for 14 days. Litter numbers were 12 to 14 pups for each experiment to assure consistency in outcomes. Two hours preceding the end of the 50% oxygen cycle at postnatal day 8 (p8), 1µl of lentivector containing VEGFA-shRNA or Luc-shRNA and was given to each group (PBS, control-luciferase, VEGA shRNA); Quantification of IVNV(B) and Avascular area (AVA) (C) in each group. (D) Weight gain of pups from postnatal day 7 (p7) to p18. (E) Rectal temperature at p18. All data are means ±S.E.M. **p<0.01 vs. PBS inj. ***p<0.001 vs. luciferase-shRNA inj.

Conclusions

1. In the rat 50/10 OIR model, knockdown of Müller cell-derived VEGFA significantly reduced IVNV compared to control Luc-shRNA or PBS injection.

References


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