Müller Cell-derived VEGF164 Knockdown Reduces Retinal Neovascularization In A Rat Model Of Retinopathy Of Prematurity

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Purpose

To determine the efficacy and safety from knockdown of Müller cell expressed vascular endothelial growth factor VEGF164 in the 50/10 model of oxygen induced retinopathy (50/10 OIR model).

Introduction

Retinopathy of prematurity (ROP) affects preterm infants and remains a leading cause of childhood blindness. The frequency is increasing in developing countries. The pathophysiology of ROP can be described by two stages: 1) delay in physiological retinal vascular development, and in cases of high oxygen at birth, some vaso-attenuation of vessels causing avascular retina and 2) later abnormal neovascularization that grows into the vitreous as intraretinal neovascularization (INVN).

Vascular endothelial growth factor (VEGF) is one of the most studied factors leading to INVN in ROP and its models. We previously reported that VEGF164 and VEGFR2 were increased in a model representative of human ROP whereas other splice variants and VEGFR1 were unaffected in the model. VEGF protein was found increased in vitreous from preterm infants who had surgery for stage 4 ROP compared to controls1. Follow the recent clinical trial testing intravitreal delivery of a broad anti-VEGF antibody in infants with severe ROP, there have been concerning reports of persist avascular (AVA) retina and vasoproliferation sometimes with total retinal detachment, even one year after treatment2.

Müller cells express VEGFA in retina. Using a relevant model of ROP, we previously reported that knockdown of overexpressed Müller cell-derived VEGFA significantly reduced INVN. 3 However, VEGFA is also vital to Müller cell survival, and Müller cells provide factors important for the survival of other cell types in the retina. VEGF164 is a prevalent isoform of VEGF involved in pathogenic angiogenesis in ROP models4.5. We asked whether inhibition of Müller cell VEGF164 would reduce INVN without interfering with physiologic retinal vascular development or affect Müller and other retinal cell types in the rat ROP model.

Methods

1. Lentivirus with shRNA to VEGF164 targeting Müller cells: VEGFA and control luciferase shRNAs were created as described6. Lentivectors with CD44 promoter and short hairpin RNA (shRNA) were used to knockdown VEGFA shRNA or VEGF164 (VEGFA.shRNA). At least two different shRNAs were created and tested for knockdown efficiency in HEK reporter cell lines for VEGFA.shRNA and VEGF164.shRNA. MC-1 cells were used to test the specificity of VEGF164.shRNA, VEGF164.shRNA and luciferase control (luc.shRNA). Uninfected cells were used as controls for luc.shRNA.

2. Animal model: As reported7, within 4 hours of birth, pups and their dams were placed into an Oxycycler, which cycled oxygen between 50% and 20% every 24 hours for 14 days. At the beginning of the 50% oxygen cycle at postnatal day 8 (p8), 1 µl of lentivector containing luc.shRNA, VEGFA.shRNA or VEGF164.shRNA was given to pups as a subretinal injection. As an additional control, PBS was injected in some eyes. All pups were harvested at p18 or p25. At least 12 pups/litter are required to assure reproducibility of features in the rat ROP model.

3. Retinal flat mount and protein: In each pup, one eye was processed as a lensed stainer retinal flat mount and the other for VEGF protein (ELISA). Retinal images were analyzed for avascular total retinal area (AVA%) and INVN% using ImageJ.

4. TUNEL assay: Eyes from groups luc.shRNA, VEGFA.shRNA, VEGF164.shRNA or PBS were fixed in 4% PFA and processed as 8 µm thick cryosections for immunohistochemical analysis of TUNEL staining. TUNEL positive cells co-labeled with TMR red and DAPI were imaged at 20X and counted at 60 micron intervals.

5. Morphometric analysis: Thickness of all retinal cell layers and total retinal thickness was evaluated in DAPI-stained cryosections obtained from groups luc.shRNA, VEGFA.shRNA, VEGF164.shRNA or PBS injected eyes.

Conclusions

1. Knockdown of retinal VEGF using shRNAs to Müller cell-derived VEGFA or the splice variant VEGF164 was possible using a lentivector gene therapy strategy.

2. In the rat 50/10 OIR model, knockdown of Müller cell-derived VEGF164 showed long-term inhibition of INVN compared to VEGF164.shRNA, control luc.shRNA or PBS injection.

3. VEGF164.shRNA was able to inhibit INVN more safely than VEGF164.shRNA as determined by effect on outer nuclear layer thickness, but more studies are indicated to determine long-term functions.

4. Future studies are indicated to determine long-term function and structure from inhibition of Müller cell-derived VEGF164 or VEGF164.

References


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