Conditional knock out of the type 2 TGF-β receptor in the mouse retina influences angiogenesis

Barbara M. Braunger1, Sarah V. Leimbeck1, Cornelia Volz1, Herbert Jägle2, Ernst R. Tamm

1 Institute of Human Anatomy and Embryology, University of Regensburg, Regensburg, Germany
2 Department of Ophthalmology, University Clinic Regensburg, Germany

Background
Central retinal vein occlusion or systemic disorders as diabetes mellitus result in proliferation of capillaries. Diabetic retinopathy is a major cause of blindness worldwide. It is well known, that Tgf-β signaling is of major importance to the development and homeostasis of retinal blood vessels (Dickson et al., 1995; Walte et al., 2009). Interestingly, the role of Tgf-β in diabetic retinopathy is still discussed controversially, as both anti- and pro-angiogenic properties have been reported (Aiello et al., 1997; Pfeffer et al., 1997; Simó et al., 2006). To learn more about the role of Tgf-β signaling for retinal angiogenesis, we generated mice with a conditional deletion of the Tgf-β receptor type 2, which is essential for Tgf-β signaling.

Methods
Floxed Tgf-β-R2 mice, with LoxP sites flanking exon 2 of the type 2 Tgf-β receptor gene, were crossed with CAG-Cre mice expressing Cre recombinase under control of a tamoxifen-responsive promoter. Activation of Cre was confirmed by using a Cre-reporter mouse strain (ROSA-Lacz), expressing β-galactosidase in the presence of Cre. β-galactosidase staining shows the localization of Cre in the tissue. Conditional deletion of Tgfbr2 was confirmed by Western blot analysis and quantitative real time RT-PCR. Retinal structures were analyzed using light microscopy (4 weeks of age) and in vivo fluorescence angiography (4 and 8 weeks of age). Animals were perfused with high molecular FITC-coupled dextran and retinal flat mounts and sagittal sections were obtained to visualize retinal vessels in detail. The expression level of different angiogenic factors was analyzed using quantitative real time RT-PCR and Western blot analysis. For statistical analysis, a student’s t-test was performed (significant: p<0.05).

Results

Tgf-β signaling affects angiogenesis

Dextran perfused retinal whole mounts of a 4 week old Tgfbr2−/− mice compared to a Tgfbr2+/+ littermate (A). The left hand side gives an overview of the superior vascular plexus of the whole retina, whereas the right hand side shows a detailed view of the three vascular plexuses in the retina.

Molecular confirmation of Tgfbr2 deletion

Tgfbr2 RNA and protein levels are downregulated: Quantitative real time RT-PCR for Tgfbr2 in the retina of Tgfbr2−/− and Tgfbr2+/+ littermates (A). Western blot analysis and densitometry for TSPRI in this eye (B) and retina (C) of a Tgfbr2−/− mouse compared to a Tgfbr2+/+ littermate. Means ± SEM are shown.

Phenotype of Tgfbr2−/− mice

Phenotype analysis on semithin sections: Light micrographs of the central retina and optic nerve heads of a 4 week old Tgfbr2−/− mouse (D, above) and their Tgfbr2+/+ (D, middle/littermate). Tgfbr2−/− animals show a persistence of the hyaloid artery (black arrowheads), which reaches from the optic nerve head through the vitreous as far as the naso/temporal parts of the lens (D, below).

Conclusions
Lack of Tgf-β signaling leads to disordered retinal angiogenesis and malformation of retinal vessels. Accordingly, the retina is hypoxic and various angiogenic factors are significantly upregulated. We succeeded in generating an animal model to study the molecular pathogenesis of retinal diseases associated with neoangiogenesis such as in diabetic retinopathy or central vein occlusions.

Acknowledgments
The authors wish to thank Silvia Babal, Angelika Pach, Margret Schimmel and Elke Stauber for their excellent technical assistance. This study was supported by DFG/Research Unit (Forshergungsbericht F041075).