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

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Background

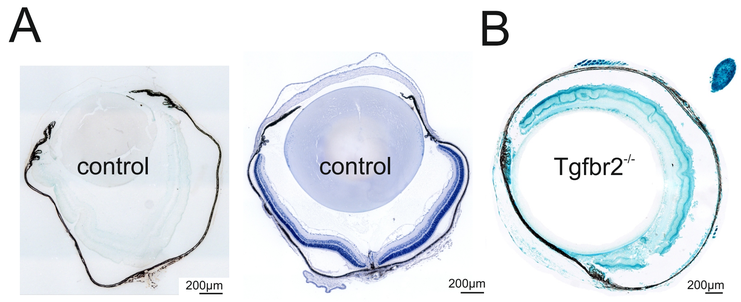
Central retinal vein occlusion or systemic disorders as diabetes mellitus result in proliferation of capillaries. Diabetic retinopathy is a major cause of blindness worldwide. It is well known, that Tgf-β signaling is of major importance to the development and homoeostasis of retinal blood vessels (Dickson et al., 1995; Walshe 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; Pfeiffer 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: $p<0.05$).

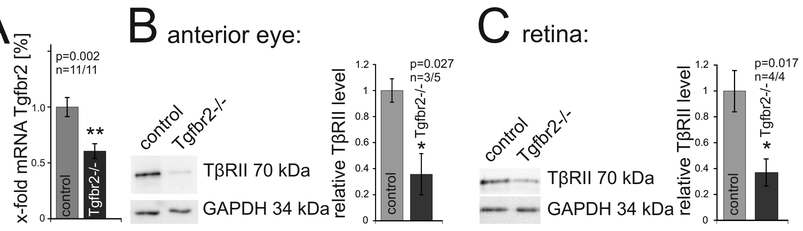
Results

Induction of Cre-recombinase using tamoxifen



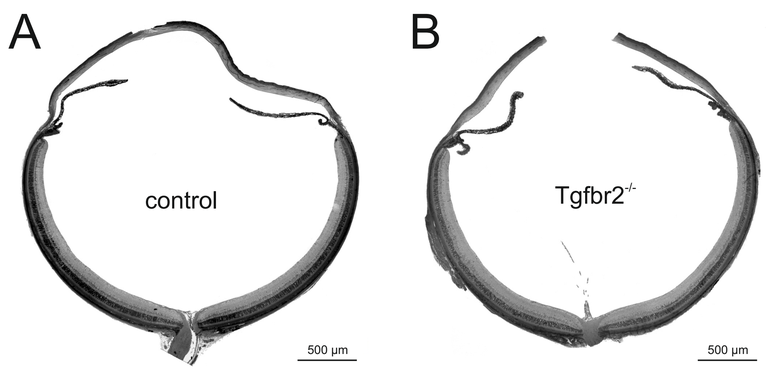
Cre-reporter mouse strain: ROSA-LacZ confirmed the induction of Cre: β-galactosidase staining of the eyes of a double transgenic *Tgfbr2*$^{-/-}$ x Rosa-LacZ mouse (**B**) and its *Tgfbr2*$^{+/+}$ x Rosa-LacZ (**A**) control littermate. To allow a better visualization of the retinal structures in the controls, we peformed additionally a haematoxylin staining (**A**, right). The blue coloured β-galactosidase staining shows the activation of the Cre-recombinase in all ocular tissues following topical tamoxifen treatment.

Molecular confirmation of *Tgfbr2* depletion

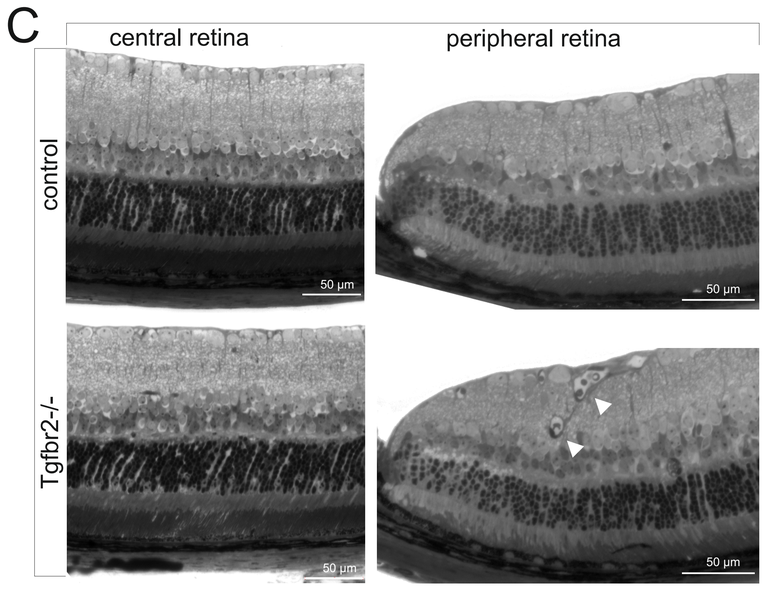


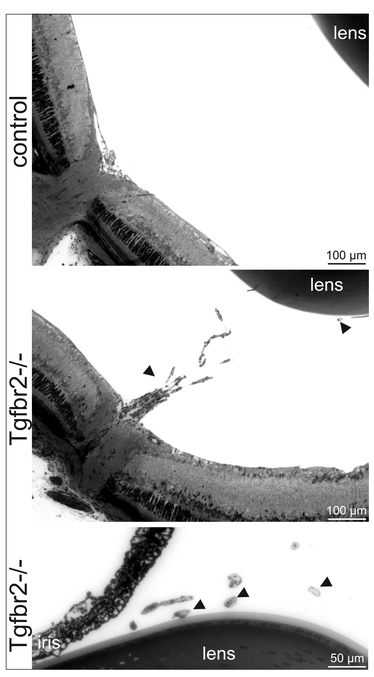
***Tgfbr2* mRNA and protein levels are downregulated:** Quantitative real time RT-PCR for *Tgfbr2* in the retina of *Tgfbr2*$^{-/-}$ and *Tgfbr2*$^{+/+}$ littermates (**A**). Western blot analysis and densitometry for TβRII in the anterior eye (**B**) and retina (**C**) of a *Tgfbr2*$^{-/-}$ mouse compared to a *Tgfbr2*$^{+/+}$ littermate. Means ± SEM are shown.

Phenotype of *Tgfbr2*$^{-/-}$ mice



Phenotype analysis on semithin sections: Lightmicrographs of the eyes and the retinae of a 4 week old control mouse (**A,C**) and its *Tgfbr2*$^{-/-}$ (**B,C**) littermate. The eyes are of comparable size and the layers of the retina (**C**) are regular. In the peripheral part of the retina, *Tgfbr2*$^{-/-}$ animals show dilated capillaries (white arrowheads).

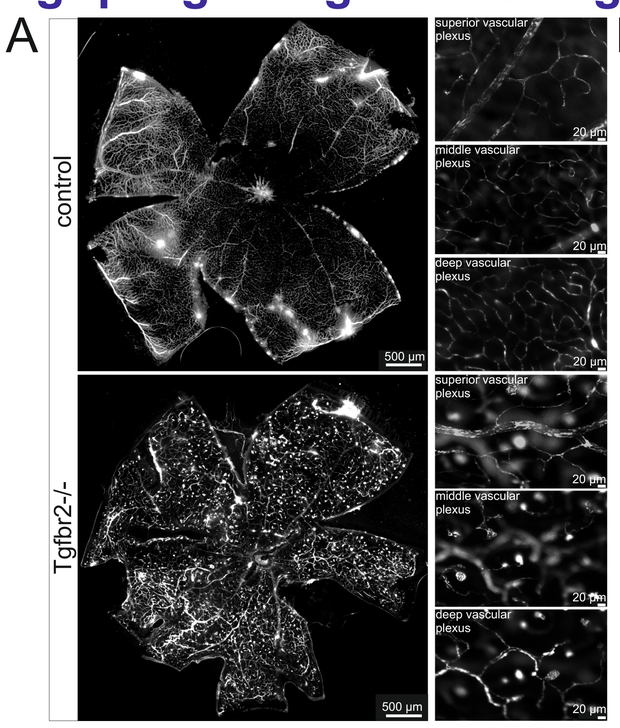




Phenotype analysis on semithin sections:
Lightmicrographs of the central retinae and optic nerve heads of a 4 week old *Tgfbr2*$^{+/+}$ mouse (**D,** above) and their *Tgfbr2*$^{-/-}$ (**D,** middle) littermate.

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 nasal/temporal parts of the lens (**D**, below).

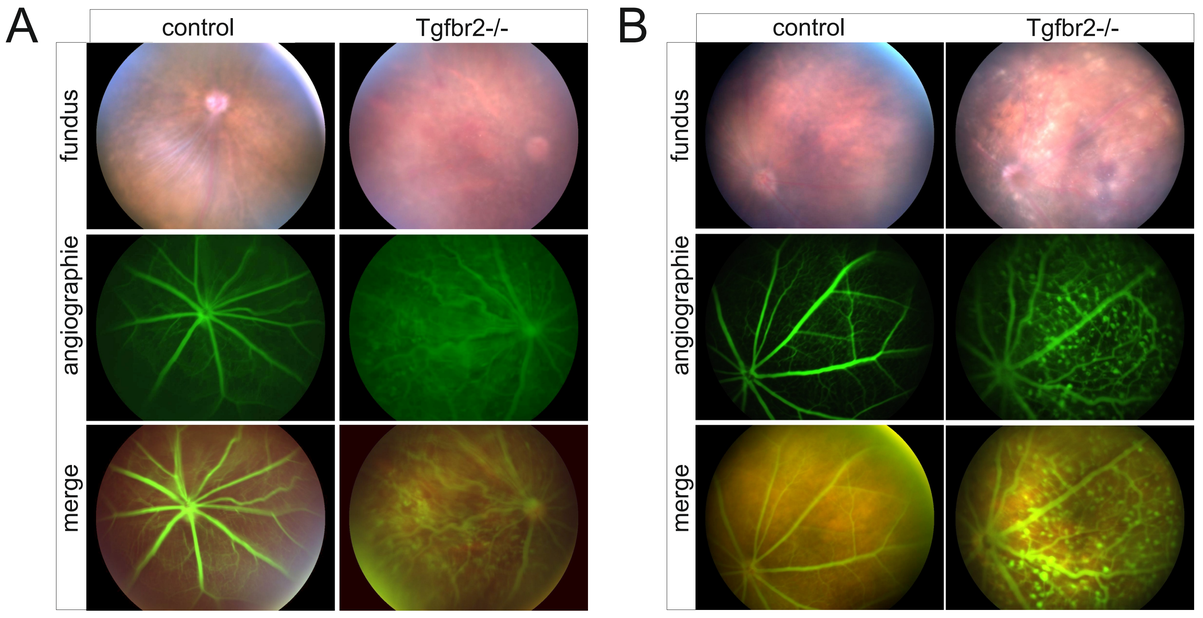
Tgf-β signaling affects angiogenesis



Dextran perfused sagittal cryosections of a 3 week old *Tgfbr2*$^{-/-}$ mouse compared to a *Tgfbr2*$^{+/+}$ control littermate (**B**). Lack of Tgf-β signaling leads to a distinct vascular phenotype involving sprouting of retinal capillaries.

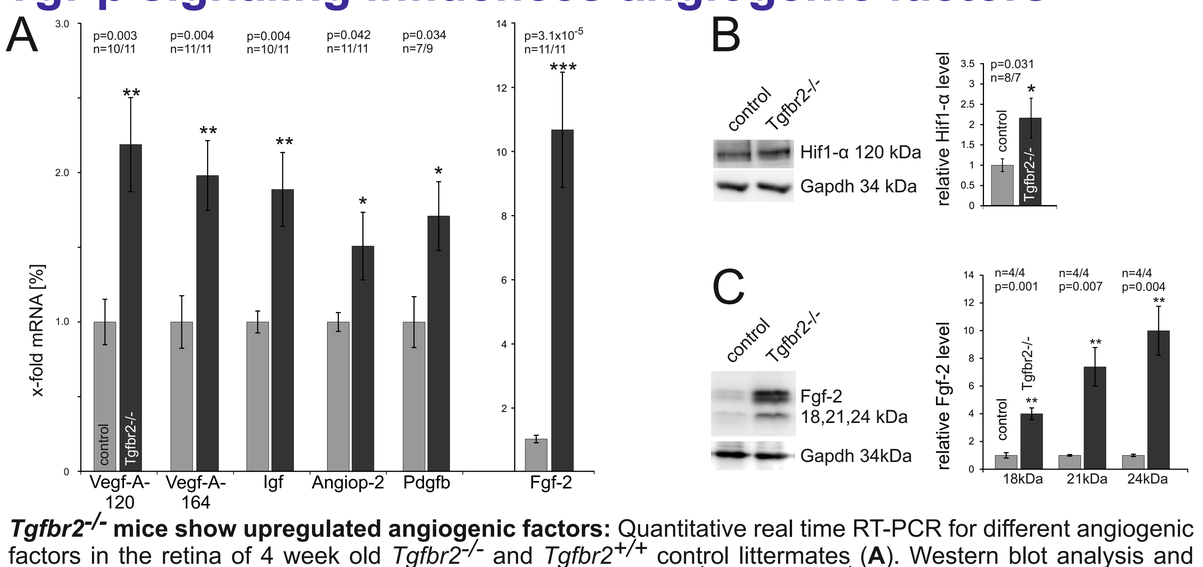
Dextran perfused retinal whole mounts of a 4 week old *Tgfbr2*$^{-/-}$ mouse compared to a *Tgfbr2*$^{+/+}$ control littermate (**A**). The left hand side gives an overview of the superior vascular plexus of the whole retina, whereas the right hand side shows a detailed view of the three vascular plexus in the retina.

Tgf-β signaling influences vascular integrity



***Tgfbr2*$^{-/-}$ animals develop distinct vascular abnormalities:** Fluorescence in vivo angiography of a 4 (**A**) and 8 (**B**) week old *Tgfbr2*$^{-/-}$ and its control *Tgfbr2*$^{+/+}$ littermate. The retinal vessels are thicker in the mutant with multiple aneurysms, especially in the 8 week old animals.
Overall, the angiographic image of the *Tgfbr2*$^{-/-}$ animal is less sharp compared to control littermate, a sign for vascular leakage, when flourescein is entering the vitreous or the tissue of the retina beside the vessels. This indicates a break down of the inner blood-retinal barrier.

Tgf-β signaling influences angiogenic factors



***Tgfbr2*$^{-/-}$ mice show upregulated angiogenic factors:** Quantitative real time RT-PCR for different angiogenic factors in the retina of 4 week old *Tgfbr2*$^{-/-}$ and *Tgfbr2*$^{+/+}$ control littermates (**A**). Western blot analysis and densitometric analysis for Hif1-α in the retina of 4 week old *Tgfbr2*$^{-/-}$ and *Tgfbr2*$^{+/+}$ control littermates (**B**). Western blot analysis and densitometric analysis for Fgf-2 in the retina of 4 week old *Tgfbr2*$^{-/-}$ and *Tgfbr2*$^{+/+}$ control littermates (**C**). Means ± SEM are shown. For statistical analysis, a student's t-test was performed (significant: $p<0.05$).

Conclusion

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.

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Acknowledgments
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