Selective Cre/lox FLT-1 ablation in RPE induces CNV: A novel transgenic murine CNV model

A451

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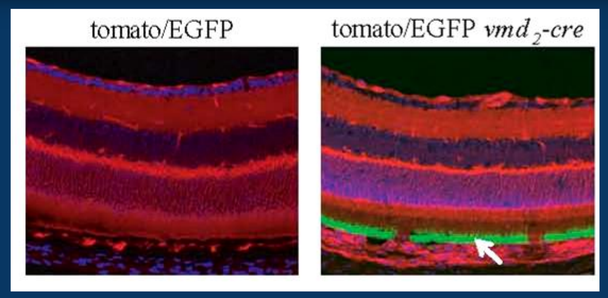
Objective

To determine whether choroidal neovascularization (CNV) is developed spontaneously, without injury, by conditional ablation of VEGF receptor-1 (Flt-1) in the retinal pigment epithelium (RPE).

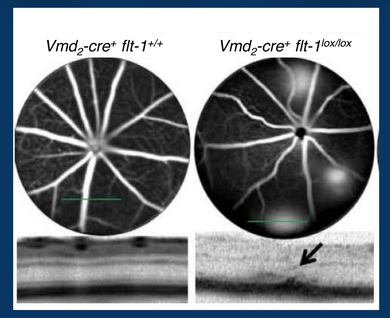
Methods

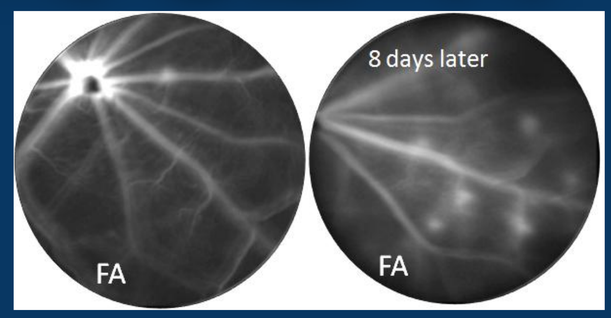
- We interbred transgenic *Vmd$_2$-cre* mice, which express Cre recombinase specifically in the RPE cells with floxed *Flt-1* mice. At 21 days to 3 months after birth, the fundi were observed *in vivo* by fluorescein angiography (FA) and indocyanine green (ICG) angiography using the Heidelberg Retina Angiograph. Histology was performed by hematoxylin and eosin (H&E) staining and transmission electron microscope (TEM).
- Cre expression in the *Vmd$_2$-cre* lines was identified by interbreeding with mT/mG mice expressing tomato/EGFP in all tissues.
- The soluable Flt-1 expression was analyzed by immunohisochemistry (IHC) and *in situ* hybridization.

Results

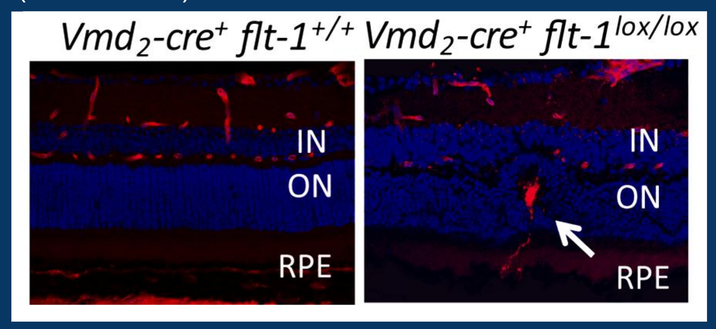


Deletion of tomato fluorescence showed that Cre expression (mosaic pattern) was restricted to RPE in transgenic mT/mG *vmd$_2$-cre$^+$* mice; (arrows highlight Cre expression).

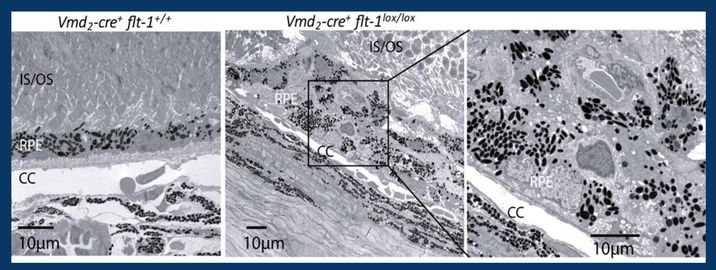




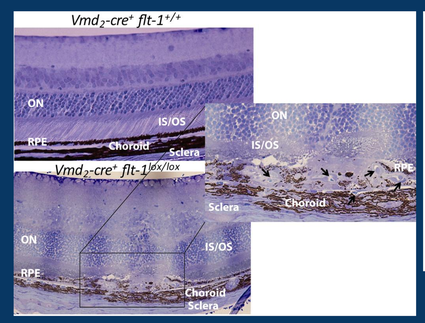
At 21 days to 3 months of age, all homozygous RPE-specific *Flt-1* knockout mice (*Cre$^+$ flt-1$^{lox/lox}$*) (18/22 eyes, 82%, *P*=1.3E-6), and about half of the hemizygous conditional *Flt-1* knockout mice (*Cre$^+$ flt-1$^{lox/+}$*) (17/42 eyes, 40%, *P*=0.009) developed CNV, which progressed over time, compared with 18% (2/22 eyes, 9%) of littermate controls (*Cre$^+$ flt-1$^{+/+}$*).



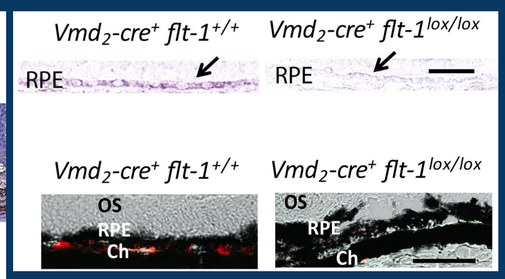
IHC images showed retinal neovessels in the above *Vmd$_2$-cre$^+$ flt-1$^{lox/lox}$* (CNV) mice at the age of 3 months.



Representative TEM images show CNV in a homozygous conditional Flt-1 knockout mouse (*Vmd$_2$-cre$^+$ flt-1$^{lox/lox}$*) compared to normal retinal architecture in a littermate control.



Toluidine blue stained images show CNV in a homozygous conditional Flt-1 knockout mouse (*Vmd$_2$-cre$^+$ flt-1$^{lox/lox}$*, 3 months old) compared to normal retinal morphology in a littermate control (arrows show the new vessels with red blood cells).



Soluble FLT-1 down-regulation was confirmed by *in situ* hybridization and IHC staining.

Conclusions

FLT-1 (soluble FLT-1) knockdown in the RPE by selective Cre/lox FLT-1 ablation can induce early stage CNV. The transgenic mice developed in this study could be potentially used as a novel murine CNV model.

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