Early Vascular and Neuronal Changes in a VEGF Transgenic Mouse Model of Retinal Neovascularization

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PURPOSE. To investigate early retinal changes in a vascular endothelial growth factor (VEGF) transgenic mouse (tr029VEGF; rhodopsin promoter) with long-term damage that mimics nonproliferative diabetic retinopathy (NPDR) and mild proliferative diabetic retinopathy (PDR).

METHODS. Rhodopsin and VEGF expression was assessed up to postnatal day (P)28. Vascular and retinal changes were charted at P7 and P28 using sections and wholemounts stained with hematoxylin and eosin or isolectin IB4 Griffonia simplicifolia. Samples were examined using light, fluorescence, and confocal microscopy.

RESULTS. Rhodopsin was detected at P5 and reached mature levels by P15; VEGF protein expression was transient,peaking at P10 to P15. In wild-type (wt) mice at P7, vessels had formed in the nerve fiber/retinal ganglion cell layer and showed a centroperipheral maturation gradient; some capillaries had formed a second bed on the vitread side of the inner nuclear layer (INL). By P28, the retinal vasculature had three mature capillary beds, the third abutting the scleral aspect of the INL. In tr029VEGF mice, capillary bed formation was accelerated compared with that in wt, with abnormal vessels extending to the scleral side of the INL by P7 and abnormally penetrating the photoreceptors by P28. Compared with P7, vascular lesions were more numerous at P28 when capillary dropout was also evident. At both stages, retinal layers were thinned most where abnormal vessel growth was greatest.

CONCLUSIONS. Concomitant damage to the vasculature and neural retina at early stages in tr029VEGF suggest that both tissues are affected, providing opportunities to examine early cellular events that lead to long-term disease.

Clinically, diabetic retinopathy (DR) has chronic progression, with early nonproliferative diabetic retinopathy (NPDR) being characterized by increased vessel permeability, microaneurysms, and capillary loss.1–16 Ensuing retinal ischemia triggers proliferative DR (PDR), with neovascularization leading to fibrovascular changes, retinal detachment, and blindness.17 Loss of cells in the neural retina as well as glial changes occur early in DR and often precede vascular disease.8–11

The usefulness of animal models for DR depends on the degree to which the pathologic features mimic those seen clinically. STZ models suffer the disadvantages of only transient and often late-onset changes with no neural loss, as well as systemic toxicity.12–14 The Ins2Akita mouse is spontaneously hyperglycemic and characterized by mild and late NPDR with some neuronal loss in the inner nuclear layer (INL) and inner plexiform layer (IPL),15 whereas the IGF-1 transgenic progresses to severe PDR with retinal detachment and cataract;16 neuronal damage has not been examined. Eye-specific VEGF transgenics have also been generated, and while some show severe and rapid progression to PDR,17–19 others display retinopathy that is relatively slow and mild (tr029VEGF).19,20

We have recently charted vascular changes in tr029VEGF from 6 weeks after birth and show progression from NPDR to mild PDR with increased permeability; pericyte and endothelial cell loss; vessel tortuosity leukostasis; and capillary blockage, dropout, and hemorrhage.20 In this study, we investigated earlier stages, from before the onset of transgene expression (embryonic day [E]18) until 4 weeks after birth, to examine the sequence of abnormal vessel formation and compare initial changes in the vasculature with those in the neural retina. Part of this work has been published in abstract form.21

MATERIALS AND METHODS

Animals and Anesthesia

Procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the Institutional Animal Ethics Committee. Fourth- and fifth-generation tr029VEGF littermates were bred as previously described.18 Mice were screened by amplification of tail genomic DNA. Anesthesia was administered with ketamine and xylazine (100 and 10 mg/kg body weight intraperitoneally [IP], respectively; Troy Laboratories Pty. Ltd., Smithfield, NSW, Australia); euthanasia was performed with pentobarbitone (200 mg/kg body weight IP; Jurox, Newcastle, NSW, Australia). Wild-type (wt) littermates served as control subjects.

Color Fundus Photography and Fluorescein Fundus Angiography

The retinal vasculature was viewed at P28 (n = 3) using color fundus photography and fluorescein fundus angiography to examine microaneurysm formation and vascular leakage, as previously described.22

RNA Extraction and cDNA Synthesis

Retinal RNA was extracted (Masterpure DNA and RNA purification kit; MC 85200) and quality and concentration checked spectrophotometri-
cally. For cDNA synthesis, 1 μg of RNA was mixed with 500 μM of oligo dT and 40 mM dNTPs, incubated at 70°C for 5 minutes with 200 units of reverse transcriptase (Superscript II; Invitrogen Life Technologies), five times first-strand buffer, 40 units of RNase inhibitor (Invitrogen Life Technologies, Sydney, Australia) and 5 mM dithiothreitol (DTT) were added and incubated (42°C, 50 minutes), followed by heat inactivation (70°C, 15 minutes). One unit of RNaseH (Invitrogen Life Technologies) was added and incubated (30°C, 20 minutes); cDNA was stored at −20°C.

Real-Time PCR Analysis
PCR reaction components for quantitation were 2 picomoles of primer, nucleic acid stain (SYBR Green), 10X reaction buffer, 2 mM MgCl₂, 0.2 mM dNTPs, 0.5 units polymerase (Platinum Taq; Invitrogen Life Technologies), and 1 μL of cDNA per 20 μL. Reactions were performed (Rotorgene 3000; Corbett Research, Mortlake, NSW, Australia), dissociation profiles were used to check single-product amplification, and random samples were sequenced for verification.

Protein Analysis
Ocular hVEGF₁₆₅ was quantified in tr029VEGF at P10, P15, P20, and P28 (n = 4 eyes per stage) using ELISA (Quantikine; R&D Systems, Minneapolis, MN).

Tissue Preparation: Sectioned Material
Eyes were marked dorsally, fixed in 4% paraformaldehyde for 2 hours, placed in 70% ethanol overnight, and wax embedded. Orientation was so as to section ventrodorsally and reveal the nasotemporal axis; 1 of every 10 sections (6-μm sections) was collected.

Series were stained with hematoxylin and eosin, rhodopsin, isolectin IB4 (Griffonia simplicifolia), or glial fibrillary acidic protein (GFAP). Sections were permeabilized (1% with Triton X-100 plus cations) in PBS, blocked with 0.3% H₂O₂ and washed in PBS. Primary antibodies were diluted in PBS Triton X-cations: rhodopsin Rho-4D2 (1:1000; the kind gift of David Hicks, INSERM, City University Hospital, Strasbourg, France), isolectin IB4 Griffonia simplicifolia (1:100; Vector Laboratories, Burlingame, CA), GFAP (1:1000; Dako, Botany, NSW Australia) for incubation overnight at 4°C. Slides were washed, incubated with streptavidin-horseradish peroxidase (50% in PBS, LSAB Kit; DakoCyto) at room temperature for 1 hour, washed, incubated in 3,3′-diaminobenzidine (DAB) substrate, washed, dehydrated, cleared, and mounted.

Wholemounts
Retinas with a dorsal orientation mark were fixed in 4% paraformaldehyde (PBS, pH 7.4, 20 minutes) and washed in PBS/cations (0.1 mM MnCl₂, 0.1 mM MgCl₂, and 0.1 mM CaCl₂ in PBS) with Triton X-100 (1%). Wholemounts were incubated in biotinylated isolectin IB4 (1:100 dilution, in PBS/cations; Vector Laboratories) at 4°C overnight, washed, incubated in a peroxidase conjugate (Extravidin; 1:150 in PBS/cations; Vector Laboratories) at 4°C overnight, followed by heat inactivation (70°C, 15 minutes). One unit of RNaseH (Invitrogen Life Technologies, Sydney, Australia) was stored at 20°C.

Blood vessel location within the different retinal layers was charted in sections stained with isolectin IB4 at P7 (n = 3) and P28 (n = 3). Low-power images from the midventral, central, and middorsal retina were captured and used as a template to map blood vessels, which were identified at high power. Cup-shaped maps of individual sections were converted to straight lines.

Wholemounts
The panretinal distribution of the retinal vasculature was examined in isolectin IB4-stained wholemounts from the vitread surface at P7 (n = 7) and P28 (n = 8) with fluorescence or confocal microscopy, and photomontages were generated. For tg029VEGF, we mapped regions of capillary dropout and the location of vascular lesions that were defined as bright fluorescent spots resembling a spectrum from small microaneurysms to larger intraretinal microvascular abnormalities (IRMAs) seen both experimentally and clinically. The numbers of vascular lesions was compared between the dorsonasal, dorsotemporal, ventro nasal and ventrotemporal retinal quadrants and between central and peripheral retinal halves.

The detailed structure of the vascular beds and vascular lesions was analyzed with confocal microscopy. Representative areas were captured in the z-plane in 1.5- to 2.0-μm steps. Color coding was used to distinguish vessels in the upper (red: nerve fiber–retinal ganglion cell layers, NFL/RGCL) middle (green), and lower (blue) capillary beds, which abutted, respectively, the vitread and scleral margins of the INL. Yellow was used for vessels that had extended into the photoreceptor outer segments (OS).

Statistics
Data were compared by using the two-sample Student’s t-test assuming unequal variances (significance P < 0.05; Excel; Microsoft, Inc., Redmond, WA).

RESULTS
Rhodopsin Expression
In wt mice, rhodopsin mRNA expression was detectable at P5 and increased thereafter (Fig. 1A); expression did not differ significantly between wt and tr029VEGF (Fig. 1A; P > 0.05). At P7 and P28, rhodopsin immunostaining was uniformly intense from the center to the periphery (Figs. 1B–1I). By P28 in both wt and tr029VEGF, an immunopositive ONL and OS could be distinguished centrally and peripherally (Figs. 1F–1I).

VEGF Expression
hVEGF₁₆₅ mRNA transcript was detected at all stages tested (Fig. 1J). In agreement with previous findings, hVEGF₁₆₅ protein expression was transient—being, respectively, 38.9 ± 3.7 and 36.6 ± 2.3 pg/mg at P10 and P15 but declining to 10.4 ± 2.4 and 15.2 ± 1.1 pg/mg at P20 and P28 (Fig. 1K).

Vascular Leakage
In wt mice at P28, the retinal vasculature was mature, having major retinal vessels that radiated from the optic disc and an evenly distributed capillary bed; fluorescein leakage was not observed (Figs. 1L). In tr029VEGF, mild changes were seen with enlarged major vessels and evidence for vascular lesions and some leakage (Figs. 1M).
Retinal Vascular Morphology and Distribution of Blood Vessels

At E18, H&E sections revealed no discernible differences in overall retinal architecture between wt and tr029VEGF (Fig. 2). At P7, IB4-stained wholemounts of both wt and tr029VEGF revealed a regular alternating pattern of radial retinal arteries and veins and an upper capillary bed that had extended to within 100 to 200 μm of the periphery (Figs. 3A, 3B, 3M, 3N). In addition, in both wt and tr029VEGF, a maturational centropapillary gradient was seen within the upper capillary bed. Centrally, the capillaries displayed a regular, open network whereas peripherally, the arrangement was more complex and the advancing front of newly formed vessels could be seen at the far periphery (Figs. 3E–H). By P28 in wt, the vasculature was mature (Figs. 3C, 3I, 3J).

Vascular lesions were present at P7 in tr029VEGF but were more numerous and prominent at P28, although their numbers were variable (Figs. 3B, 3D, 3G, 3H, 3K, 3L, 3M–P). At P7, the number of vascular lesions was 54 ± 31, did not favor any retinal quadrant (dorsonasal: 14 ± 11; dorsoventral: 16 ± 20; ventronasal: 10 ± 6; ventrotemporal: 13 ± 15; P > 0.05) but were concentrated more centrally than peripherally (central: 41 ± 20; peripheral: 11 ± 16; P < 0.05). By P28, the number of vascular lesions had increased (Figs. 3D, 3K, 3L, 3O, 3P) to 153 ± 128, did not favor any retinal quadrant (dorsonasal: 30 ± 42; dorsoventral: 42 ± 45; ventronasal: 33 ± 22; ventrotemporal: 47 ± 33; P > 0.05) and showed no significant difference centrally compared with peripherally (central: 72 ± 73; peripheral: 81 ± 58; P > 0.05). Centrally, some regions showed capillary dropout (Figs. 3O, 3P).

IB4 staining in retinal wholemounts showed that vessel formation in tr029VEGF was accelerated compared with wt. At P7 in wt, the upper capillary bed displayed a regular arrangement and initial sprouting toward the inner retina. The first growth of vessels to form the middle bed was seen as bulbous sprouts connected to parent capillaries in the upper bed by a thin bridge (Figs. 4A, 4B). Areas with more mature, continuous vessels were also seen (Fig. 4C). However, some peripheral regions had only an upper capillary bed and had yet to start forming the middle bed (Fig. 4D). In tr029VEGF, some retinal regions appeared normal (Fig. 4E) but, in contrast to wt, most had begun to form the middle bed. Sprouts forming the middle bed were larger and more bulbous compared with wt (Figs. 4E, 4F). In other regions, abnormal vessels had formed and extended farther toward the outer retina (Figs. 4G, 4H). Some vascular lesions were discrete, and the upper and middle beds appeared similar to normal (Fig. 4G). In others, vascular lesions were large and tortuous, and both the upper and middle beds showed dropout (Fig. 4H). Vascular lesions were not observed in the upper capillary bed.

By P28 in wt, three (upper, middle, and lower) capillary beds had formed and appeared as a regular and nonoverlapping array (Fig. 4I). A possible sequence of vascular lesion formation is shown in Figures 4J–P. In tr029VEGF, some regions appeared normal (not shown), whereas others had slight capillary enlargements in the middle and lower beds (Fig. 4J). Vascular lesions were more common than at P7. Some were continuous between the middle and lower beds (Figs. 4K, 4L). Others formed a continuous structure between the lower bed and the OS (Figs. 4K, 4L) and appeared to enlarge by folding back on themselves (Figs. 4M, 4N). Vascular lesions were larger in the lower bed and in the OS compared with those in the inner bed (Figs. 4O, 4P). Furthermore, capillary beds appeared
normal away from the vascular lesions (Figs. 4J, 4L) but suffered dropout immediately above and surrounding them (Figs. 4F–P). Reconstructions through the z-plane (Fig. 5; Movies 6D–10) revealed that vascular lesions comprised continuous capillaries that were highly tortuous and had a diameter of 27.8 ± 0.3 μm (range, 10–46 μm). H&E sections allowed three-dimensional mapping of capillaries in relation to the retinal layers and confirmed the acceleration of capillary bed formation in tr029VEGF (Figs. 6A–H). In tr029VEGF, abnormal vessels also invaded and disrupted the ONL and OS as well as the retinal pigment epithelium (Figs. 6D, 6H, 6L–K). GFAP staining of Müller cell processes showed no difference between wt and tr029VEGF at P7, with immunostaining being restricted to the vicinity of the inner limiting membrane (ILM; Figs. 6L, 6M). By P28, the OLM was also stained in both wt and tr029VEGF although immunoreactivity was greater in tr029VEGF and increased in regions associated with vascular lesions (Figs. 6N, 6O).

Changes in the thickness of the retinal layers were quantitated from H&E-stained sections. At P7 in tr029VEGF, regions both with and without vascular lesions, were significantly reduced, the effect being greater where lesions were present. In central regions with vascular lesions, the overall thickness of the retina was considerably reduced compared with (wt: 190.2 ± 9.3 μm; tg: 160.8 ± 7.2 μm; P < 0.05) with significant reductions in the NFL, INL, and ONL (P < 0.05; Figs. 7C, 7D, 7F, 7H, 7J). The overall thickness of central retinal regions lacking vascular lesions was also significantly less than in the wt (tg: 169.6 ± 5.3 μm; P < 0.05), a difference attributable to a thinner INL (P < 0.05; Figs. 7A, 7B, 7F, 7J). The same pattern was seen in the peripheral retina lacking vascular lesions, with overall retinal thickness being less in tr029VEGF than in wt (tg: 166.6 μm ± 3.9; wt: 189.9 ± 3.8 μm; P < 0.01), again with the difference being attributable to a thinner INL andNFL (P < 0.01; Figs. 7D, 7F, 7J).

At P28 in tr029VEGF, there was a similar pattern of thinning associated with the vascular lesions, but different retinal layers were affected compared with P7. For central retina with vascular lesions, overall retinal thickness was reduced compared with wt with losses attributable to reductions in the IPL and OS (tg: 126.5 ± 16.6 μm; wt: 180.5 ± 3.1 μm; P < 0.05; Figs. 7M, 7O, 7S, 7T). Overall thickness of central retinal regions lacking vascular lesions was also reduced compared with wt (tg: 168.1 ± 5.5 μm; P < 0.05) with differences being attributed to reductions in NFL, INL, and ONL (P < 0.05; Figs. 7K, 7L, 7S, 7T). The overall thickness of peripheral retina which lacked retinal lesions did not differ compared with wt (tg: 150.16 μm; wt: 150.5 ± 2.7 μm; P > 0.05; Fig. 7T).

**Discussion**

Early changes within the retinal vasculature and neural retina of tr029VEGF were mild and coincided with early hVEGF expression. Initially, vascular lesions were small and resembled microaneurysms that were visible clinically but then progressed to larger structures with contorted capillaries that resembled IRMAs. Thinning of the neural retina coincided with the formation of vascular lesions, but not all retinal layers were affected equally. Furthermore, although retinal regions with vascular lesions experienced the most damage, regions distant from lesions were also somewhat affected.

Our results support previous evidence of an early but transient upregulation in hVEGF165 protein in tr029VEGF, which, as we showed, coincides with early damage to the vasculature and neural retina. Such damage persists up to 24 weeks, even in the absence of continued protein expression, suggesting that an initial "spike" in hVEGF165 is sufficient to induce long-term changes characteristic of NPDR. The greater vascular and retinal damage seen in this model of DR is presumably related to sustained and high levels of VEGF expression, as previously shown in human DR.

VEGF models differ from DR because normal vessel growth is toward the transgene source within the photoreceptors in the outer retina rather than toward the inner retina and vitreous. However, although the direction of abnormal vessel growth might be different in our VEGF model compared with human DR, we showed that the process of new vessel formation and associated complications was the same. Furthermore, the damage that we and others have observed within photoreceptors is also seen in human DR, suggesting that these cells are particularly vulnerable. We have also recently shown that changes within the retinal vasculature in tr029VEGF are accompanied by structural damage to the choroid that mimics that in DR, suggesting that pathophys...
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**Figure 4.** Retinal wholemounts at P7 (A–H) and P28 (I–P) stained with IB4 showing the upper (NFL/RGCL: red), middle (vitreous side of the INL: green), and lower (scleral side of the INL: blue) capillary beds in wt (P7; A–D, P28: I) and tr029VEGF (P7; E–H, P28: J–P) animals. (A–D) In wt animals at P7, the upper capillary bed (red) had formed and had thin bridges (green, arrow) tipped by bulbous sprouts (green, open arrow) which extended toward the INL (A, B). In some instances, (C, arrowbeads) capillaries in the middle bed (green) were continuous rather than ending. In the far periphery, the upper capillary bed (red) lacked capillary sprouting toward the INL. (E–H) Micrographs showing proposed sequence of vascular lesion formation at P7. Capillary beds showing initial bulbus structures sprouting toward the INL were numerous compared with wt (E, green, open arrow) and were connected to the upper capillaries (red) by thin bridges (E, green, arrow). Continuous, but swollen, capillaries in the middle capillary bed were also seen (E, F, green, arrowbeads). In addition, vascular lesions extended toward the outer retina (G, H, blue). In G, the vascular lesion (blue) was relatively confined, whereas the upper (red) and middle (green) capillary beds appeared normal (>). (H) The vascular lesion (blue) was more extensive, and both the upper (red) and middle (green) capillary beds showed capillary dropout. (I) At P28 in wt animals, the upper (red), middle (green), and lower (blue) vascular beds formed a regular, mostly nonoverlapping array. (J–P) Sequence of micrographs showing presumed progression of vascular lesions at P28 in tr029VEGF, from discrete swellings to large complex structures. (J) Normal-appearing region with the exception of mild vascular lesions appearing as swellings along capillaries in the lower bed (blue, arrowbeads) accompanied by further folding and extension into the photoreceptor layer (yellow, arrowbeads). Scale bars, 100 μm.

Although rhodopsin is first expressed in the murine retina at P5, the inner retina has yet to fully mature, and the ONL and OS are not yet distinct. Also, the central-to-peripheral gradients of cell division and synaptogenesis are not complete until P11. Our immunohistochemical analysis of rhodopsin expression and the thickness of the OS is similar peripherally and centrally and that at P7. Taken together, the data suggest that elevated hVEGF concentrations are likely to be equal across the retina. Nevertheless, in tr029VEGF at P7, growth of vessels toward the outer retina is accelerated centrally compared with peripherally and in addition, vascular lesions appear first centrally. The pattern matches the central-to-peripheral and inner-to-outer sequence of normal vessel formation observed in mammals and suggests that older vessels are more susceptible than younger ones. Of note, the initial central location of vascular lesions in tr029VEGF is similar to DR in which neovascular changes consistently originate close to the optic disc in 90% of cases.
of microaneurysms in humans revealed limited tortuosity,\textsuperscript{25} larly susceptible.\textsuperscript{56,57} human DR donor tissue, photoreceptors appear to be particu-
larly large lesions in tr029VEGF were highly tortuous ves-
cells and resembled the IRMAs seen clinically.\textsuperscript{40–42} Our obser-
tr029VEGF and by analogy also in clinical DR. In humans, microaneurysms are often blocked by blood cells.\textsuperscript{25,43} Presum-
ably, the tortuous nature of the vascular lesions in tr029VEGF also contributes to capillary blockage in addition to the leuko-
stasis that we have observed in this model.\textsuperscript{20,44,45}

Although, by far, most studies on DR have focused on retinal vascular changes, early observations in diabetic patients have also revealed neural losses.\textsuperscript{10,47} More recently, attention has returned to the neural retina with evidence for early functional changes as revealed by abnormal ERG recordings, thinning of the different retinal layers, and neural apopto-
sis.\textsuperscript{10,11,48–54} Our findings show that overall retinal thickness was re-
duced at P7 and further by P28 and support the clinical scen-
ario, although not all layers were affected equally. At P7, reductions occurred within the NFL/RGCL, INL, and ONL sug-
gesting losses within each nuclear layer, but by P28, no signif-
ificant differences were seen between wt and tr029VEGF, indic-
ating recovery. However, thinning of nuclear layers could reflect the same number of cells but with smaller sizes and therefore increased density or changed extracellular space, a feature that is likely, since edema occurs in DR.\textsuperscript{1,2}

By P28, however, the IPL was thinner than in wt animals, suggesting reductions in dendritic tree size and/or synaptogen-
esis. Indeed, ERG recordings at early stages of DR reveal changes in oscillatory potentials indicative of degraded ama-
crine cell function.\textsuperscript{55} However, the most striking loss was within the OS which were considerably shortened. Similarly, in human DR donor tissue, photoreceptors appear to be particu-
larly susceptible.\textsuperscript{56,57}

We have yet to determine the mechanisms whereby ele-
vated VEGF leads to early onset of both vascular and neural damage. Nevertheless, we showed that vascular and neural damage coincided chronologically, since we saw changes in both components very soon after transgene expression. Dam-
age was also spatially correlated, since regions with vascular lesions displayed more neural damage than those without.

![Figure 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932935/)

**Figure 5.** Z-stacks of vascular lesions in Figures 4F (A), 4L (B), 4N (C), and 4P (D). Vascular lesions were not simple enlargements of capillaries but rather were composed of continuous and contorted capillaries that were folded on themselves that resemble microaneu-
rysms and IRMAs seen in clinical DR. Scale bar, 50 μm.

![Figure 6](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932935/)

**Figure 6.** Sectioned retinas stained with isoelectin B4 at P7 and P28 in wt (A, C) and tr029VEGF (B, D) showing the location of vessels in relation to retinal layers. Vessels formed the upper capillary bed in theNFL/RGCL (red arrows) and the middle and lower capillary beds on the vitread (green arrows) and sclera (blue arrows) sides of the INL. In addition, in tr029VEGF, vessels extended into the OS (yellow arrow). Some vessels in the outer retina in tr029VEGF appeared abnor-
amal and tortuous (B, D; blue arrows). (E–H) Representative recon-
structions of three sections from dorsal (top row), central (middle row), and ventral (bottom row) retina depicting blood vessels in the upper (red dots), middle (green dots), and lower (blue dots) capillary beds and vessels extending into the photoreceptors (yellow dots) at P7 (E, F) and P28 (G, H) in wt (E, G) and tr029VEGF (F, H). Lines in rows of red dots indicate the extent of retinal tissue, (i.e., to the limbus). (I–K) H&E-stained sections of vascular lesions showing disruption of the outer retina and RPE. (L–O) Sectioned retinas stained with GFAP at P7 (L, M) and P28 (N, O) in wt (L, N) and tr029VEGF (M, O), showing location of GFAP-immunopositive Müller cell processes. At P7, no differences were detected between wt and tr029VEGF, with staining confined to the ILM. By P28 in wt animals, staining was also present in the OLM but in tr029VEGF, was upregulated and localized immunopo-
sitive Müller cell processes, which extended into the photoreceptor layer and were associated with vascular lesions (arrows). (D, V, N, T) Dorsal, ventral, nasal, and temporal, respectively. Scale bars: (A–D, I) 100 μm; (J, K) 20 μm; (L–O) 50 μm.
Furthermore, neural retina lacking vascular lesions was also affected, but less so, suggesting long-range effects possibly from diffusion of soluble VEGF. Transgenic models such as tr029VEGF provide an opportunity to examine cellular changes that mimic those seen in DR and to determine the extent to which damage cascades within the vasculature impinge on the neural retina and vice versa.

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References
