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Long-term expression of glial cell line-derived neurotrophic factor slows, but does not stop retinal degeneration in a model of retinitis pigmentosa

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Abstract

Retinitis pigmentosa is a group of diseases in which one of hundreds of mutations causes death of rod photoreceptor cells and then cones gradually die from oxidative damage. As different mutations cause rod cell death by different mechanisms, mutation-specific treatments are needed. Another approach is to use a neurotrophic factor to promote photoreceptor survival regardless of the mechanism of cell death, and previous studies have demonstrated encouraging short-term results with gene transfer of glial cell line-derived neurotrophic factor (GDNF). We generated *rd10* mice with doxycycline-inducible expression of GDNF in photoreceptors (*Tet/IRBP/GDNF-rd10* mice) or retinal pigmented epithelial cells (*Tet/VMD2/GDNF-rd10* mice). In doxycycline-treated *Tet/IRBP/GDNF-rd10* mice, there was a 9.3×10^4 -fold increase in *Gdnf* mRNA at P35 and although it decreased over time, it was still increased by 9.4×10^3 -fold at P70. *Gdnf* mRNA was increased 4.5×10^2 -fold in doxycycline-treated *Tet/VMD2/GDNF-rd10* mice at P35 and was not significantly decreased at P70. GDNF protein levels were increased about 2.3-fold at P35 and 30% at P70 in *Tet/IRBP/GDNF-rd10* mice, and in *Tet/VMD2/GDNF-rd10* mice they were increased 30% at P35 and not significantly increased at P70. Despite the difference in expression, *Tet/IRBP/GDNF-rd10* and *Tet/VMD2/GDNF-rd10* mice had comparable significant increases in outer nuclear layer thickness and mean photopic and scotopic ERG b-wave amplitudes compared with *rd10* mice at P35 which decreased, but was still significant at P70. Compared with *rd10* mice, *Tet/IRBP/GDNF-rd10* and *Tet/VMD2/GDNF-rd10* mice had comparable significant improvements in cone density at P50 that decreased, but were still significant at P70. These data indicate that despite a large difference in expression of GDNF, *Tet/IRBP/GDNF-rd10* and *Tet/VMD2/GDNF-rd10* provide comparable slowing of photoreceptor degeneration, but cannot stop the degeneration.

Keywords

apoptosis; cone photoreceptors; electroretinograms

Retinitis pigmentosa (RP) is a group of diseases caused by mutations in hundreds of different genes that cause death of rod photoreceptors. After rods die, there is a marked reduction in oxygen utilization resulting in high levels of oxygen in the outer retina causing progressive oxidative damage to cones and gradual cone cell death (Yu *et al.* 2000; Shen *et al.* 2005; Komeima *et al.* 2006). One treatment strategy is to use gene therapy to replace mutant genes in patients with recessive RP, but this would not apply to patients with dominant RP, and as causative mutations for recessive RP can occur in hundreds of genes, each gene replacement therapy will apply to only a small percentage of RP patients. Therefore, there is strong incentive to develop treatments that provide benefit irrespective of the underlying mutation. Neurotrophic factors are agents that promote survival of neurons that are under duress from a wide variety of insults and thus may provide a treatment that is applicable to all patients with RP.

Increased expression of glial cell line-derived neurotrophic factor (GDNF) in the retina slows the death of rod photoreceptors in models of retinal degeneration and reduces retinal degeneration from severe oxidative stress (McGee *et al.* 2001; Dong *et al.* 2007; Dalkara *et al.* 2011; Del Rio *et al.* 2011). In addition, high levels of GDNF reduced levels of reactive oxygen species in the retina after intraocular injection of paraquat (Dong *et al.* 2007). The combined activities of slowing mutation-induced death of rods and providing protection against oxidative damage caused us to hypothesize that sustained expression of GDNF would prevent death of cones and thereby provide stable cone function over time.

One way to test this is by gene transfer using AAV vectors. Although viral vectors, non-viral gene transfer, or another mechanism to provide sustained delivery of GDNF would be needed to apply this approach in patients, the transgenic approach provides advantages to test proof-of-concept in animal models. This is because after subretinal injection of an AAV vector, transduction of photoreceptors and retinal pigmented epithelium (RPE) cells is limited to the area of the retina detached by the injection and thereby exposed to the vector. As the area of detachment created by subretinal injection can vary considerably from mouse to mouse, this adds to variability and generally causes an underestimation of the optimal achievable effects. Prior to embarking on gene transfer, it is useful to utilize transgenic mice with tissue-specific promoters to drive expression of the transgene of interest in a uniform, predictable manner to try and determine the maximum achievable effects. We have utilized the tet/on inducible expression system (*Tet*), which utilizes the *reverse tetracycline transactivator (rtTA)* coupled to a tissue-specific promoter and the *tetracycline response element (TRE)* coupled to a gene of interest to test the effects of over-expressing various proteins in photoreceptors or retinal pigmented epithelium (Ohno-Matsui *et al.* 2002; Nambu *et al.* 2004; Oshima *et al.* 2005; Nambu *et al.* 2005; Usui *et al.* 2009). The *interphotoreceptor retinoid binding protein (IRBP)* promoter drives expression in both rod and cone photoreceptors (Yokoyama *et al.* 1992). We previously generated *IRBP/rtTA-TRE/GDNF (Tet/IRBP/GDNF)* double transgenic mice and demonstrated that they are resistant to oxidative damage-induced retinal degeneration (Dong *et al.* 2007). In RP, rods die from the underlying mutation and even with expression of GDNF in rods one would expect that the number of rods would decrease over time. Thus, one source of GDNF production would decrease, which could lead to a substantial reduction in GDNF production although it should

still be produced in surviving cones which could be ideal for cone survival. We reasoned that production of GDNF in RPE cells which are adjacent to photoreceptors, but not subject to the degeneration, would allow us to test the effect of sustained high-level expression of GDNF and would be useful for comparison. The *VMD2* (*vitelliform macular dystrophy two*) promoter drives expression specifically in RPE cells (Esumi *et al.* 2004) and therefore we used *Tet/VMD2/GDNF* double transgenic mice to test the effects of expression of GDNF in RPE cells.

Materials and methods

Mutant *rd10* mice with inducible expression of GDNF in photoreceptors or RPE cells

Mice were treated in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Research and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Doxycycline-inducible expression of several different proteins in the retina using transgenic mice in which the *IRBP* promoter or the *VMD2* promoter drives expression of *rtTA* (*IRBP/rtTA* or *VMD2/rtTA* mice) has previously been reported (Ohno-Matsui *et al.* 2002; Okoye *et al.* 2003; Nambu *et al.* 2004; Oshima *et al.* 2004; Dong *et al.* 2007; Usui *et al.* 2009). The same strategy was used to over-express GDNF in *rd10*^{+/+} mice (Jackson Laboratories, Bar Harbor, ME, USA) with an inherited retinal degeneration. *TRE/GDNF*(+/+) mice (Dong *et al.* 2007) and *rd10*^{+/+} mice were used in an elaborate mating scheme to generate *TRE/GDNF*(+/+)-*rd10*^{+/+} mice, *IRBP/rtTA*(+/+)-*rd10*^{+/+} mice, and *VMD2/rtTA*(+/+)-*rd10*^{+/+} mice. These mice were crossed to generate *IRBP/rtTA*(+/-)-*TRE/GDNF*(+/-)-*rd*^{+/+} mice or *VMD2/rtTA*(+/-)-*TRE/GDNF*(+/-)-*rd*^{+/+} with doxycycline-inducible expression of GDNF in photoreceptors or RPE cells.

Genotyping of mice

Genotyping was done by PCR of tail DNA using the following primers: *TRE/GDNF* (5'-CAGATACATCCACACCTTTTAGC-3' and 5'-GCTGTTTTGACCTCCATAGAAGA-3') and *IRBP/rtTA* or *VMD2/rtTA* (5'-GTTTACCGATGCCCTTGAATTGACGAGT-3' and 5'-GATGTGGCGAGATGCTCTTGAAGTCTGGTA-3'). To distinguish homozygous *rd10*, heterozygous *rd10*, and wild-type mice, the PCR fragment generated with 5'-CTTTCTATTCTCTGT CAGCAAAGC-3' and 5'-CATGAGTAGGGTAAACATGGTCT G-3' was digested with *NarI*.

Measurement of GDNF expression

Tet/IRBP/GDNF-rd10 and *Tet/VMD2/GDNF-rd10* pups were given subcutaneous injections of 0.35 mg/g body weight of doxycycline at P11, P14, and P17. After weaning male and female mice were given drinking water containing 2 mg/ml of doxycycline. *GDNF* mRNA was measured in retina and choroid by real-time RT-PCR at P35, P50, and P70 using primers specific for GDNF (5'-AA CCCAGAGAATTCCAGA-3' and 5'-AGCCGCTGCAGTACCTA AAA-3'). Cyclophilin A was used as a control for normalization (5'-CAGACGCCACTGTCGCTTT-3' and 5'-TGTCTTTGGAAC TTTGTCTGCAA-3'). Retinas and choroids were dissected and RNA was isolated using RNeasy kits (Qiagen Inc., Chatsworth, CA, USA). After treatment with DNase I (Invitrogen,

Carlsbad, CA, USA), 1 µg of RNA was incubated with SuperScript III reverse transcriptase (Invitrogen) and 5 µM oligo(dT) primer (Invitrogen). Samples of cDNA were aliquoted and stored at –80°C. RT-PCR was performed using a Rotor-Gene Q (QIAGEN). An ELISA kit (Promega, San Luis Obispo, CA, USA) was used to measure GDNF protein levels in retinal and choroidal homogenates and values were normalized to protein concentration (Bio-Rad Protein Assay Kit, Bio-Rad, Hercules, CA, USA).

Measurement of outer nuclear layer thickness

Outer nuclear layer (ONL) thickness was measured as previously described (Komeima *et al.* 2007). The 12:00 pole of the eye was marked before euthanasia and 10 µm frozen sections cut perpendicular to the 12:00 meridian were stained with hematoxylin and eosin. Image analysis (Image-Pro Plus software, Media Cybernetics, Silver Spring, MD, USA) was used to measure ONL thickness at six locations: 25%, 50%, and 75% of the distance between both the superior pole and the optic nerve and the inferior pole and the optic nerve (S1, S2, S3 and I1, I2, I3, respectively).

Recording of electroretinograms

An Espion electroretinogram (ERG) Diagnosys machine (Diagnosys LLL, Littleton, MA, USA) was used to record ERGs in female *Tet/IRBP/GDNF-rd10* and *Tet/VMD2/GDNF-rd10* mice as previously described (Komeima *et al.* 2006). For scotopic recordings, mice were dark adapted overnight, and for photopic recordings, mice were adapted to background white light at an intensity of 30 cd/m² for 10 min. The mice were anesthetized with an intraperitoneal injection of ketamine hydrochloride (100 mg/kg body weight) and xylazine (5 mg/kg body weight). Pupils were dilated, platinum electrode was placed on each cornea, and a reference electrode was placed subcutaneously in the anterior scalp between the eyes. With body temperature maintained at 39°C, light stimuli were provided by a Ganzfeld bowl illuminator ensuring equal illumination of the eyes, and responses from each eye were simultaneously recorded. Scotopic ERGs were recorded at 11 intensity levels of white light that ranged from –3.00 to 1.40 log cd-s/m². Photopic ERGs were recorded at three intensity levels of white light ranging from 0.60 to 1.40 log cd-s/m² with a 30 cd/m² background. Six measurements were averaged for each flash intensity.

Measurement of cone cell density

Male and female *Tet/IRBP/GDNF-rd10* and *Tet/VMD2/GDNF-rd10* mice were killed and eyes were removed and fixed in 4% paraformaldehyde for 3 h or overnight at 4°C. After washing with phosphate-buffered saline, the cornea, iris, and lens were removed. A small cut was made at 12:00 in the retina for future orientation and the retinas were dissected from the eye cup and any adherent RPE was removed. Retinas were blocked with 10% normal goat serum in phosphate-buffered saline containing 0.05% Tween (PBST) for 30 min and incubated for 1 h in 1 : 100 rhodamine-conjugated peanut agglutinin (Vector Laboratories, Burlingame, CA, USA) in PBST. After rinsing in PBST, retinas were flat mounted and examined by light microscopy. Images were digitized and the number of cones within four 230 × 230 mm squares located 0.5 mm superior, inferior, temporal, and nasal to the center of the optic nerve was determined using image analysis.

Statistical analysis

Data are expressed as means $\pm$ SE. Statistical analysis was performed using analysis of variance (ANOVA) with Dunnett's correction for multiple comparisons, and $p < 0.05$ was considered significant.

Results

Expression of GDNF in eyes of *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice

Mothers of *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice were given water containing 2 mg/ml of doxycycline and the pups were given subcutaneous injections of 0.35 mg/g body weight doxycycline at P11, P14, and P17. After weaning, the mice were given water containing 2 mg/ml of doxycycline. At P35, there was a 9.3×10^4 -fold increase in *Gdnf* mRNA in the eyes of *Tet/IRBP/GDNF-rd10* mice and a 4.5×10^2 -fold increase in the eyes of *Tet/VMD2/GDNF-rd10* mice compared with control *rd10* mice (Fig. 1a). Compared with the P35 level, there was a significant reduction in *Gdnf* mRNA in the eyes of *Tet/IRBP/GDNF-rd10* mice at P50 and P70, but it was still very high, 3.6×10^4 -fold and 9.4×10^3 -fold higher than in control *rd10* mice. There was no significant decrease in *Gdnf* mRNA in the eyes of *Tet/VMD2/GDNF-rd10* mice between P35 and P50, but regardless *Gdnf* mRNA was substantially higher in *Tet/IRBP/GDNF-rd10* mice at each time point.

Despite the enormous increases in *Gdnf* mRNA in the eyes of *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice, there was a modest, significant increase in GDNF protein from 2.7 ng/mg total protein in eyes of *rd10* or C57BL/6 wild-type mice to 6.1 ng/mg in *Tet/IRBP/GDNF-rd10* mice or 3.5 ng/mg in *Tet/VMD2/GDNF-rd10* mice at P35 (Fig. 1b). Compared with the levels at P35, GDNF levels decreased at P50 and P70 in *Tet/IRBP/GDNF-rd10* mice, but were still significantly greater than those in *rd10* or wild-type C57BL6 mice. GDNF levels also decreased over time in eyes of *Tet/VMD2/GDNF-rd10* mice and were still significantly higher than *rd10* and wild-type mice at P50, but not P70.

Rod photoreceptor survival and function in *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice

Compared with *rd10* mice, there were significant increases in mean ONL thickness, a measure of rod photoreceptor survival, at four locations in the retinas of *Tet/IRBP/GDNF-rd10* mice and at six locations in the retinas of *Tet/VMD2/GDNF-rd10* mice at P35 with no difference between the two (Fig. 2). These values are about 16% of those seen in wild-type mice. There were significant increases in mean scotopic ERG b-wave amplitudes indicating greater rod function in *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice compared with *rd10* mice at P35, P50, and P70 (Fig. 3a). At P35, b-wave amplitudes were 15% of normal at the lowest stimulus intensity and 10% at the highest intensity, and at P70 they were 10% (lowest stimulus intensity) and 5% (highest stimulus intensity).

Cone photoreceptor survival and function in *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice

There were significant increases in mean photopic ERG b-wave amplitudes indicating greater cone function in *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice

compared with *rd10* mice at P35–P70 (Fig. 3b). At P35, photopic b-wave amplitudes were 23% of normal at low stimulus intensity and 15% at high stimulus intensity, and at P70 they were 17% (low intensity) and 12% (high intensity). Compared with *rd10* mice, mean cone density was significantly greater in each of the four quadrants of the retina at P50 and P70 in *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice (Fig. 4). Cone survival was greatest in the superior quadrant where it was 19% of normal at P50 and 13% at P70, whereas in the temporal quadrant, it was 9% of normal at P50 and 7% at P70.

Discussion

In this study, we used a transgenic approach to over-express GDNF in photoreceptors or RPE cells of *rd10* mice. Levels of *Gdnf* mRNA in the eye were very high when the IRBP promoter was used to drive expression, and although there was a 10-fold reduction between P35 and P70, the level was still 9.3×10^3 -fold above baseline at P70. The VMD2 promoter drove lower level expression of *Gdnf* mRNA, but it was still more than 4.5×10^2 -fold higher than baseline at P35 and remained stable through P70. In previous studies, we have found that compared with the VMD2 promoter, the IRBP promoter drives higher expression of transgenes in the eye, but the magnitude of the *Gdnf* mRNA expression with either promoter was somewhat surprising. As the IRBP promoter drives expression in rods and cones, we anticipated that unless increased levels of GDNF completely prevented photoreceptor cell death, that IRBP-induced expression would decrease over time and this is what occurred. Conversely, the residual high expression of GDNF at P70 indicates that there were still surviving cones and they were healthy enough to support robust transcriptional activity. We were gratified to see that VMD2-induced expression of *Gdnf* mRNA in RPE cells remained stable over time.

Relative to the large increases in *Gdnf* mRNA, increases in GDNF protein were small in doxycycline-treated *Tet/IRBP/GDNF-rd10* mice but still more than 2-fold above baseline at P35. This was reduced to a 30% increase at P70 that was significantly above levels in control mice. Compared with GDNF levels in *Tet/IRBP/GDNF-rd10* mice, those in doxycycline-treated *Tet/VMD2/GDNF-rd10* mice were substantially less, but significantly greater than those in control mice at P35 and P50. Even if the ELISA-measured levels are considerably less than the actual levels of GDNF, the results are surprising and suggest a marked perturbation in translation compared to transcription in both photoreceptors and RPE cells of *rd10* mice. It is also surprising that GDNF levels decrease over time in the eyes of both *Tet/IRBP/GDNF-rd10* and *Tet/VMD2/GDNF-rd10*. We anticipated that as RPE cells do not degenerate in *rd10* mice, GDNF protein levels like *Gdnf* mRNA levels would remain stable over time in *Tet/VMD2/GDNF-rd10*. It appears that the relative defect in translation worsens over time in both RPE and photoreceptors, despite the greater survival of RPE cells.

Compared with untreated *rd10* mice, there was significant preservation of rod and cone structure and function in *Tet/IRBP/GDNF-rd10* and *Tet/VMD2/GDNF-rd10* mice at P35, P50, and P70 with no identifiable difference between the two despite the substantially higher levels of GDNF in eyes of *Tet/IRBP/GDNF-rd10* mice. This suggests that a maximal survival-promoting effect is achieved by the levels of GDNF obtained in *Tet/VMD2/GDNF-rd10* mice and that higher levels provide no additional benefit. Although this maximal

survival-promoting effect is impressive, it is clear that there is continued death of rods and cones between P35 and P70, and that the residual rod and cone function at P70 is a small fraction of that seen in wild-type mice (roughly 5–10% for rods and 12–15% for cones). These data suggest that increased expression of GDNF in photoreceptors or RPE cells definitely increases the threshold for apoptosis in photoreceptors of *rd10* mice, but does not prevent apoptosis from ultimately occurring. As the mechanism of cell death differs in rods and cones, and cones die more slowly suggesting that the insult is less severe in cones than in rods, we had hypothesized that even if transgenic expression of GDNF did not prevent rod cell death, it would prevent cone cell death. Our data do not support this hypothesis. In contrast with antioxidant treatments that provide substantially better protection for cones (Komeima *et al.* 2006, 2007; Usui *et al.* 2009), over-expression of GDNF provided only slightly better maintenance of survival and function for cones than for rods. This suggests that GDNF protects against mutation-induced cell death as well as oxidative damage-induced cell death.

Our data suggest that gene- or cell-based therapy to provide high levels of GDNF may not be an ideal stand-alone treatment for RP; however, increasing the threshold for apoptosis may still be useful if combined with treatments targeting the mechanism of cell damage. The mechanism of rod cell death varies depending upon the mutation, but cones die from oxidative damage regardless of the underlying mutation (Komeima *et al.* 2006, 2007; Usui *et al.* 2009) Thus, combining increased expression of GDNF with treatments that protect against oxidative damage deserves further investigation.

Acknowledgments

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Abbreviations used

ERG	electroretinograms
GDNF	glial cell line-derived neurotrophic factor
IRBP	interphotoreceptor retinoid binding protein
ONL	outer nuclear layer
PBST	phosphate-buffered saline containing 0.05% tween
RP	retinitis pigmentosa
RPE	retinal pigmented epithelium
rtTA	reverse tetracycline transactivator
TRE	tetracycline response element
VMD2	vitelliform macular dystrophy two

References

- Dalkara D, Kolstad KD, Guerin KI, Hoffmann NV, Visel M, Klimczak RR, Schaffer DV, Flannery JG. AAV mediated GDNF secretion from retinal glia slows down retinal degeneration in a rat model of retinitis pigmentosa. *Mol Ther*. 2011; 19:1602–1608. [PubMed: 21522134]
- Del Rio P, Irmier M, Arango-Gonzalez B, et al. GDNF-induced osteopontin from Muller glial cells promotes photoreceptor survival in Pde6brd1 mouse model of retinal degeneration. *Glia*. 2011; 59:821–832. [PubMed: 21360756]
- Dong A, Shen J, Krause M, Hackett SF, Campochiaro PA. Increased expression of glial cell line-derived neurotrophic factor protects against oxidative damage-induced retinal degeneration. *J Neurochem*. 2007; 103:1041–1052. [PubMed: 17935603]
- Esumi N, Oshima Y, Li Y, Campochiaro PA, Zack DJ. Analysis of the VMD2 promoter and implication of E-Box binding factors in its regulation. *J Biol Chem*. 2004; 279:19064–19073. [PubMed: 14982938]
- Komeima K, Rogers BS, Lu L, Campochiaro PA. Antioxidants reduce cone cell death in a model of retinitis pigmentosa. *Proc Natl Acad Sci USA*. 2006; 103:11300–11305. [PubMed: 16849425]
- Komeima K, Rogers BS, Campochiaro PA. Antioxidants slow photoreceptor cell death in mouse models of retinitis pigmentosa. *J Cell Physiol*. 2007; 213:809–815. [PubMed: 17520694]
- McGee LH, Abel H, Hauswirth WW, Flannery JG. Glial cell line derived neurotrophic factor delays photoreceptor degeneration in a transgenic rat model of retinitis pigmentosa. *Mol Ther*. 2001; 4:622–629. [PubMed: 11735347]
- Nambu H, Nambu R, Oshima Y, Hackett SF, Wiegand SJ, Yancopoulos G, Zack DJ, Campochiaro PA. Angiopoietin 1 inhibits ocular neovascularization and breakdown of the blood-retinal barrier. *Gene Ther*. 2004; 11:865–873. [PubMed: 15042118]
- Nambu H, Umeda N, Kachi S, Oshima Y, Nambu R, Campochiaro PA. Angiopoietin 1 prevents retinal detachment in an aggressive model of proliferative retinopathy, but has no effect on established neovascularization. *J Cell Physiol*. 2005; 204:227–235. [PubMed: 15648096]
- Ohno-Matsui K, Hirose A, Yamamoto S, et al. Inducible expression of vascular endothelial growth factor in photoreceptors of adult mice causes severe proliferative retinopathy and retinal detachment. *Am J Pathol*. 2002; 160:711–719. [PubMed: 11839592]
- Okoye G, Zimmer J, Sung J, et al. Increased expression of BDNF preserves retinal function and slows cell death from rhodopsin mutation or oxidative damage. *J Neurosci*. 2003; 23:4164–4172. [PubMed: 12764104]
- Oshima Y, Oshima S, Nambu H, et al. Increased expression of VEGF in retinal pigmented epithelial cells is not sufficient to cause choroidal neovascularization. *J Cell Physiol*. 2004; 201:393–400. [PubMed: 15389527]
- Oshima Y, Oshima S, Nambu H, et al. Different effects of angiopoietin 2 in different vascular beds in the eye; new vessels are most sensitive. *FASEB J*. 2005; 19:963–965. [PubMed: 15802489]
- Shen J, Yan X, Dong A, Petters RM, Peng YW, Wong F, Campochiaro PA. Oxidative damage is a potential cause of cone cell death in retinitis pigmentosa. *J Cell Physiol*. 2005; 203:457–464. [PubMed: 15744744]
- Usui S, Komeima K, Lee SY, et al. Increased expression of catalase and superoxide dismutase 2 reduces cone cell death in retinitis pigmentosa. *Mol Ther*. 2009; 17:778–786. [PubMed: 19293779]
- Yokoyama T, Liou G, Caldwell RB, Overbeek PA. Photoreceptor-specific activity of the human interphotoreceptor (IRBP) promoter in transgenic mice. *Exp Eye Res*. 1992; 55:225–233. [PubMed: 1426058]
- Yu DY, Cringle SJ, Su EN, Yu PK. Intraretinal oxygen levels before and after photoreceptor loss in the RCS rat. *Invest Ophthalmol Vis Sci*. 2000; 41:3999–4006. [PubMed: 11053305]

Fig. 1.

Doxycycline-induced expression of glial cell line-derived neurotrophic factor (GDNF) mRNA and protein in the eyes of *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice. At P11, P14, and P17, *Tet/IRBP/GDNF-rd10* mice or *Tet/VMD2/GDNF-rd10* mice were given subcutaneous injections of 0.35 mg/g body weight doxycycline and then were given drinking water containing 2 mg/ml of doxycycline from P21 through P70. At P35, P50, and P70, mice were killed and in some mice, retinas and retinal pigmented epithelium/choroid were dissected, and mRNA (a) and protein (b) levels for human GDNF were measured. (a) Real-time RT-PCR was performed using primers specific for human GDNF or Cyclophilin A. There were very high levels of *Gdnf* mRNA in the eyes of *Tet/IRBP/GDNF-rd10* mice at P35 that decreased over time but were still high at P70. Levels of *Gdnf* mRNA were not as high in *Tet/VMD2/GDNF-rd10* mice, but were quite stable over time. Each bar represents the mean ($\pm$ SEM). ** $p < 0.01$ versus P35. # $p < 0.01$ versus P50. (b) ELISA on retina and retinal pigmented epithelium/choroid homogenates showed significant increases in GDNF protein in both *Tet/IRBP/GDNF-rd10* and *Tet/VMD2/GDNF-rd10* mice at P35, but the magnitude of the increases was less than that seen for *Gdnf* mRNA. Each bar represents the mean ($\pm$ SEM). * $p < 0.05$ and ** $p < 0.01$ versus *rd10* mice. # $p < 0.01$ versus *Tet/VMD2/GDNF-rd10*.

Fig. 2.

Rod photoreceptor survival in *Tet/IRBP/(GDNF)-rd10* mice and *Tet/VMD2/GDNF-rd10* mice. At P35, mice were killed and ONL thickness was measured in *Tet/IRBP/GDNF-rd10* and *Tet/VMD2/GDNF-rd10* mice treated with doxycycline. Compared with *rd10* mice, there were significant increases in mean ONL thickness, a measure of rod photoreceptor survival, at four locations in the retinas of *Tet/IRBP/ GDNF-rd10* mice and at six locations in the retinas of *Tet/VMD2/ GDNF-rd10* mice with no difference between the two. Each bar represents the mean ($\pm$ SEM). * $p < 0.05$ and ** $p < 0.01$ versus *rd10* mice.

Fig. 3.

Rod and cone photoreceptor function in *Tet/IRBP/(GDNF)-rd10* mice and *Tet/VMD2/GDNF-rd10* mice. At P35, P50, P60, and P70, ERGs were done in *Tet/IRBP/GDNF-rd10* mice, and *Tet/VMD2/GDNF-rd10* mice treated with doxycycline. (a) There were significant increases in mean scotopic ERG b-wave amplitudes indicating greater rod function in *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice compared with *rd10* mice at each time point. (b) There were significant increases in mean photopic ERG b-wave amplitudes indicating greater cone function in *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice compared with *rd10* mice at each time point. Each bar represents the mean ($\pm$ SEM). * $p < 0.05$ and ** $p < 0.01$ versus *rd10* mice. # $p < 0.05$ and ## $p < 0.01$ versus *Tet/IRBP/GDNF-rd10*. The number of the eyes at P35, P50, P60, and P70 for each of the groups were: (a) *rd10* (20, 20, 20, 20), *Tet/IRBP/GDNF-rd10* (20, 20, 18, 18), *Tet/VMD2/GDNF-rd10* (24, 18, 20, 20); (b) *rd10* (20, 20, 24, 20), *Tet/IRBP/GDNF-rd10* (20, 20, 18, 16), *Tet/VMD2/GDNF-rd10* (24, 18, 20, 20).

Fig. 4.

Cone photoreceptor survival in *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice. At P50 (a) and P70 (b), mice were killed and cone cell density was measured in *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice treated with doxycycline. Compared with *rd10* mice, mean cone density was significantly greater in each of the four quadrants of the retina at each time point in *Tet/IRBP/GDNF-rd10* mice and *Tet/VMD2/GDNF-rd10* mice. Each bar represents the mean ($\pm$ SEM). * $p < 0.01$ versus *rd10* mice.