Levels of Vascular Endothelial Growth Factor-A165b (VEGF-A165b) are Elevated in Experimental Glaucoma

Citation
Ergorul, Ceren, Arjun Ray, Wei Huang, Diane Darland, Zhonghui K. Luo, and Cynthia L. Grosskreutz. 2008. Levels of vascular endothelial growth factor-A165b (VEGF-A165b) are elevated in experimental glaucoma. Molecular Vision 14: 1517-1524.

Published Version
http://www.molvis.org/molvis/

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:4881145

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
Levels of vascular endothelial growth factor-A165b (VEGF-A165b) are elevated in experimental glaucoma

Ceren Ergorul,1 Arjun Ray,1 Wei Huang,1 Diane Darland,2 Zhonghui K. Luo,1 Cynthia L. Grosskreutz1

1Howe Laboratory of Ophthalmology, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA; 2University of North Dakota, Department of Biology, Grand Forks, ND

Purpose: Although ischemia has previously been suggested to contribute to the pathogenesis of glaucoma, neovascularization is not implicated in glaucoma. Because vascular endothelial growth factor-A (VEGF-A) is a key mediator in neovascularization response, we investigated the levels of the major pro-angiogenic (VEGF-A164) and anti-angiogenic VEGF-A subtypes (VEGF-A165b) in the retina during experimental glaucoma.

Methods: Glaucoma was induced unilaterally in rats by injecting 1.9 M hypertonic saline solution in the episcleral veins. The contralateral eye served as the control. The intraocular pressure (IOP) of each eye was measured via Tonopen in conscious rats. Eyes were enucleated either on the 5th or the 10th day of elevated IOP. Whole retinal lysates were separated by SDS–PAGE and transferred to PVDF membranes. Levels of VEGF-A164 and VEGF-A165b were analyzed by western blotting using specific antibodies. In a different group of rats, retinal ganglion cells were retrogradely labeled by injecting Fluorogold in the superior colliculus a week before the induction of glaucoma. After the eyes were enucleated on the fifth day of elevated IOP, posterior eye cups were sectioned using a cryostat. Levels and localization of VEGF-A164 and VEGF-A165b were examined in retinal sections by immunohistochemistry.

Results: VEGF-A164 levels remained unchanged between the control and glaucomatous retinas after five days (p=0.341) and 10 days of elevated IOP (p=0.117). The presence of the anti-angiogenic VEGF-A isoform has not been previously reported in the rat. An antibody specific to VEGF-A165b detected the anti-angiogenic protein in the rat retina. VEGF-A165b levels were significantly increased (2.33±0.44 fold, p=0.014) in the glaucomatous retinas compared to those in controls after five days of elevated IOP. VEGF-A165b levels were not different (p=0.864) between the control and glaucomatous retinas following 10 days of elevated IOP. Expression of both VEGF-A164 and VEGF-A165b were observed in the retinal ganglion cells (RGC) and inner nuclear layer (INL).

Conclusions: Five day elevation of IOP leads to an increase in the anti-angiogenic VEGF-A165b levels but not in the pro-angiogenic VEGF-A164 levels in the glaucomatous retina. VEGF-A165b levels return to baseline after 10 days of elevated IOP, and VEGF-A164 levels remain unchanged. We speculate that the short-term elevation of VEGF-A165b levels and/or the unchanged levels of VEGF-A164 contribute to the lack of neovascularization in the glaucomatous retina.

Glaucoma is a neurodegenerative disease of retinal ganglion cells (RGC) that leads to blindness. Although the most prominent risk factor for RGC death in glaucoma is elevated intraocular pressure (IOP), the sequence of events by which IOP causes RGC death still remains largely unknown. One possible mechanism that is elevated IOP can induce abnormalities in blood flow in the glaucomatous eye. In open-angle glaucoma patients, abnormal vascular autoregulation has been observed in the inferior temporal retinal artery, the central retinal artery, the circulation of the optic nerve head, the choroid, and the perifoveal macular capillaries [1-8]. It has been suggested that dysregulation of blood flow may lead to decreased vascular perfusion in the retina and in the optic nerve head, resulting in an hypoxic response [9,10].

In the classical view of hypoxia, the ischemic tissue compensates for a decrease in oxygen levels by forming new blood vessels, a process known as neovascularization [11]. VEGF-A is a key mediator in neovascularization in ischemic retinopathies [12-14]. There are several VEGF-A isoforms expressed from a single gene via alternative splicing [15,16]. Among these, VEGF-A165 is the most abundantly expressed pro-angiogenic isoform in the retina [17]. More recently, anti-angiogenic sister isoforms of VEGF-A have also been identified [18-20]. For example, VEGF-A165b, an anti-angiogenic human VEGF-A isoform, has been shown to inhibit VEGF-A induced neovascularization in the mouse retina following ischemia [21].

There are only a few studies that have examined VEGF-A in glaucoma. VEGF levels were shown to be increased in the plasma of glaucoma patients when compared to that of healthy controls [22] and in the aqueous humor of glaucoma patients when compared to their plasma VEGF levels [23]. Despite these findings, neovascularization is not implicated...
in glaucoma, and the role of VEGF-A has not been examined in the glaucomatous retina.

If ischemia contributes to the pathogenesis of glaucoma, why is there no neovascularization in glaucoma? To answer this apparent paradox, we investigated the levels of pro-angiogenic VEGF-A164 (the rat version of VEGF-A165) and anti-angiogenic VEGF-A165b (the rat version of VEGF-A165b) in normal and glaucomatous retinas after a short-term (five day) and an intermediate-term (10 day) elevation of IOP. Because of the lack of neovascularization in glaucoma, we hypothesized that the levels of VEGF-A165b but not VEGF-A164 would be increased in the glaucomatous retina.

METHODS

Subjects: Male rats (retired breeder Brown Norway; 300-450 g; n=16) were used for the study. Rats had ad libitum access to food and water during the study and were kept on a 12 h illumination cycle. All animal related procedures were performed in accordance with the statement for the use of animals in research released by the Association for Research in Vision and Ophthalmology.

Retrograde labeling of retinal ganglion cells: Rats (n=4) were anesthetized with an intraperitoneal injection of 1.5 mg/kg of acepromazine maleate, 7.5 mg/kg of xylazine, and 75 mg/kg of ketamine (Webster Veterinary Supply, Sterling, MA). Following shaving of the head, each rat was placed in a stereotaxic instrument. The skin covering the skull was exposed and leveled. Next, for each hemisphere, a 30-gauge stainless steel needle was lowered into the superior colliculus at 5.3 mm posterior to the bregma, 1.5 mm lateral to the midline, and 4.8 mm ventral to the skull surface. Using a 5 μl syringe (Hamilton, Reno, NV), 2 μl of Fluorogold solution (3% in PBS with 10% DMSO; Fluorochrome, Denver, CO) was injected over 10 min into each hemisphere. Following the injections, the skin was sutured. Rats were allowed to recover for a week before glaucoma was induced experimentally.

Experimental induction of glaucoma: To elevate IOP, hypertonic saline solution (1.9 M) was unilaterally injected in the episcleral veins as described by Morrison and colleagues [24]. The contralateral eye of the rat served as the control. A maximum number of three injections that were two weeks apart were performed in the absence of IOP elevation. Rats that did not have an elevation of IOP after the third surgery were excluded from the study.

Intraocular pressure measurements: IOPs were measured with a TonoPen XL tonometer (Medtronic Ophthalmics, Jacksonville, FL) in conscious rats [25]. Measurements were taken between 10 AM and 2 PM. Before the first hypertonic saline injection, baseline IOPs for both eyes were measured for each rat. Following glaucoma inducing surgery, IOPs were measured three times a week. On each measurement day, an average of 15 readings was calculated for each eye. This study investigated a five day (n=6) and a 10 day elevation of IOP (n=6).

Tissue preparation: Rats were sacrificed by CO2 inhalation either after five days or 10 days of elevated IOP. For western blotting, retinas were isolated from eyes obtained after five days and 10 days of elevated IOP (six pairs each). Retinas were placed in 200 μl of 1 mM of EDTA/EGTA/DTT, 10 mM of Hepes (pH=7.6), 0.5% Igepal (Sigma Chemical Co., St. Louis, MO), 42 mM of KCl, 5 mM of MgCl2, 1 mM of PMSF, and a tablet of protease inhibitors (Complete Mini, Roche Diagnostics, Mannheim, Germany). After retinas were sonicated and incubated for 15 min on ice, samples were spun at 21,000 rpm at 4 °C for 30 min. Retinal proteins were quantified by spectrophotometry using the Bio-Rad D, Protein Assay (Bio-Rad Laboratories, Hercules, CA).

For immunohistochemistry, four pairs of eyes that were enucleated after five days of elevated IOP were fixed with 4% paraformaldehyde for 20 min at room temperature. These eyes were previously back-labeled with Fluorogold. Next, the posterior eye cups were isolated and fixed with 4% paraformaldehyde for an additional 40 min at room temperature. After posterior eye cups were cryoprotected overnight in graded sucrose dilutions, they were placed in the optimal cutting temperature compound (Tissue-tek, Miles Diagnostic Division, Elkhart, IN) and were sectioned 16 μm thick using a cryostat.

Western blotting: Retinal proteins isolated after either five days or 10 days of elevated IOP were separated on Tris-HCl Ready-Gels (Bio-Rad Laboratories, Hercules, CA). Recombinant rat VEGF-A164 protein (25–250 ng; R&D Systems, Minneapolis, MN) was also loaded as a positive control in certain experiments. Proteins separated by SDS–PAGE were then transferred to polyvinylidene difluoride membranes (Immobilon-P; Millipore, Billerica, MA) for 1 h. After the membrane was blocked for 1 h at room temperature with 2% ECL Advance Blocking Agent (GE Healthcare, Piscataway, NJ) in Tris-buffered saline with Tween (TBS-T), it was incubated at 4 °C overnight with a primary antibody. The primary antibodies used in this study were as follows: rabbit polyclonal anti-VEGF (1:50; Santa Cruz Biotechnology, Santa Cruz, CA), mouse monoclonal anti-VEGF165B (1:1,000; Abcam, Cambridge, MA), and mouse monoclonal anti-α-tubulin (1:100,000; Sigma, Saint Louis, MO). The next day, membranes were incubated for 1 h at room temperature with peroxidase-conjugated secondary antibodies. Goat anti-rabbit IgG (1:10,000–1:40,000; Jackson ImmunoResearch, West Grove, PA) and goat anti-mouse IgG (1:20,000–1:100,000; Jackson ImmunoResearch) were the secondary antibodies used in the study. Both of these antibodies had minimal cross-reaction to rat serum proteins. Next, membranes were processed with ECL Advance Western Blotting Detection Kit (GE Healthcare, Piscataway, NJ) and...
Levels of VEGF-A retinas: transparency to see the overlap of different fluorophores in a then used Adobe Photoshop to layer the images and apply to the BX51 to image the retinal sections for fluorescence. We in conjunction with the DP70 color camera (Olympus) affixed Alexa Fluor 594. We used DPController 1.2.1.108 (Olympus) for Fluorogold and N41004 HQ Texas Red (Chroma) for fluorophore were 11006v2 Gold (Chroma, Rockingham, VT) respectively. Excitation/emission filter cubes used for a given our retinal sections for a total magnification of 200X or 400X, objective lenses through a 10X ocular or camera lens to image 20X (Olympus) and UPlanApo 0.85 NA 40X (Olympus, Center Valley, PA).

Immunohistochemistry: Retinal sections from four pairs of eyes were blocked for 1 h at room temperature in 4% normal goat serum and 0.3% Triton-X 100 in 1X PBS, pH 7.4. Sections were incubated with the primary antibody overnight at 4 °C. Some sections were incubated in blocking solution without the primary antibody and were used as negative controls. The primary antibodies and the dilutions used in the study were mouse monoclonal anti-VEGF (20 μg/ml; Sigma) and mouse monoclonal anti-VEGF165B (1:500; Abcam). The following day, sections were incubated with the Alexa Fluor 594-conjugated goat anti-mouse secondary antibody (1:500; Invitrogen, Carlsbad, CA) for 1 h at room temperature. After the sections were treated with Prolong Gold anti-fade reagent, staining was visualized using an Olympus BX51 microscope (Olympus, Center Valley, PA).

On our BX51 microscope, we used UPlanApo 0.70 NA 20X (Olympus) and UPlanApo 0.85 NA 40X (Olympus) objective lenses through a 10X ocular or camera lens to image our retinal sections for a total magnification of 200X or 400X, respectively. Excitation/emission filter cubes used for a given fluorophore were 11006v2 Gold (Chroma, Rockingham, VT) for Fluorogold and N41004 HQ Texas Red (Chroma) for Alexa Fluor 594. We used DPController 1.2.1.108 (Olympus) in conjunction with the DP70 color camera (Olympus) affixed to the BX51 to image the retinal sections for fluorescence. We then used Adobe Photoshop to layer the images and apply transparency to see the overlap of different fluorophores in a given section area.

RESULTS

Levels of VEGF-A164 do not change in the glaucomatous retina: Average peak IOP was (mean±SEM) 40.7±1.9 mmHg and 43.1±0.8 mmHg for the five-day (n=6) and 10-day (n=6) groups that were used in the western blot (WB) analysis, respectively. Average peak IOP was 43.4±0.8 mmHg for the other five-day group used for the immunohistochemistry (IHC) analysis (n=4).

In western blots, anti-VEGF antibody detected a 45 kDa band corresponding to the VEGF-A164 dimer in all retinas and in the positive control brain (Figure 1). VEGF-A164 levels remained unchanged between the control and glaucomatous retinas after five days (p=0.341, n=6; Figure 1A,C) and 10 days of elevated IOP (p=0.117, n=6; Figure 1B,C). Using this well characterized antibody [26,27], the VEGF-A164 monomer was not detected in the retina or in the brain.

In VEGF-A164 IHC, there was some nonspecific staining in the blood vessels in the RGC layer and INL of the negative control retinas (Figure 2B,C). VEGF-A164 staining did not differ between the normal (Figure 2E) and glaucomatous retinas (Figure 2H). VEGF-A164 expression was localized to the RGC and the cells in the INL of both groups (Figure 2E-H). In the RGC layer, VEGF-A164 staining colocalized with the retinal ganglion cell marker, Fluorogold (Figure 2F,I). Also, VEGF-A164 levels did not differ between the normal and glaucomatous retinas, which are consistent with the WB results.

Anti-VEGF-A165b antibody does not recognize VEGF-A164: Because it had been predicted that VEGF-A164 and VEGF-A165b are highly homologous in amino acid sequence [18], we first investigated whether the anti-VEGF-A165b antibody would also recognize VEGF-A164. To test this possibility, we immunoblotted different concentrations (25 ng, 100 ng, and 250 ng) of the recombinant rat VEGF-A164 protein with the anti-VEGF-A165b antibody. Whereas the anti-VEGF-A165b antibody did not recognize the VEGF-A164 protein at any concentration, it recognized two bands around 22.4 and 45 kDa in a pair of control and glaucomatous retinas corresponding to the monomer and dimer forms of VEGF-A165b, respectively (Figure 3A). Next, we stripped the

![Figure 1](http://www.molvis.org/molvis/v14/a181/1519)
membrane and subsequently immunoblotted with the anti-VEGF antibody. A 22.4 kDa VEGF-A164 monomer was detected at all concentrations (Figure 3B), confirming the presence of VEGF-A164 recombinant protein in the same membrane. The dimer form was not observed with the recombinant rat VEGF-A164 protein. The combination of these findings indicated that the anti-VEGF-A165b antibody does not recognize VEGF-A164 and that VEGF-A165b is expressed in the rat retina.

**VEGF-A165b levels are increased in the glaucomatous retina:**
Following five days of elevated IOP, the anti-VEGF-A165b antibody detected bands around 22.4 and 45 kDa in all retinas, which represent the monomer and dimer forms of VEGF-A165b, respectively (Figure 4A). Whereas VEGF-A165b dimer levels remained unchanged between the control and glaucomatous retinas (p=0.273, n=6), VEGF-A165b monomer levels were significantly increased in the glaucomatous retinas compared to those in controls (2.33±0.44 fold, p=0.014, n=6) (Figure 4A,B). However, following 10 days of elevated IOP, there was no change in levels for the VEGF-A165b dimer (p=0.483, n=6) or for the VEGF-A165b monomer (p=0.864, n=6) between the control and glaucomatous retinas.

**Figure 2.** Immunohistochemical analysis of VEGF-A164 expression in the glaucomatous retina after five days of elevated IOP. **A-C:** Negative control. Some non-specific staining of blood vessels in the RGC and the INL was observed. **D-F:** VEGF-A164 staining of the normal retina (n=4). VEGF-A164 was present in the RGC and the INL. **G-I:** VEGF-A164 staining of the glaucomatous retina (n=4). Staining was detected in the RGC and INL. VEGF-A164 levels did not differ between the normal and glaucomatous retinas.

**Figure 3.** Anti-VEGF-A165b antibody does not recognize VEGF-A164. **A:** Incubation with VEGF-A165b antibody. This antibody recognizes the VEGF-A165b monomer (22.5 kDa) and dimer (45 kDa) in control and glaucomatous retinas (first two lanes). VEGF-A165b antibody does not recognize 25 ng, 100 ng, or 250 ng of VEGF-A164 recombinant protein in the same membrane (last three lanes). **B:** Incubation of the same membrane with the anti-VEGF antibody after stripping. The anti-VEGF antibody recognizes VEGF-A164 recombinant protein at all concentrations.
A positive control was the brain while the negative control was VEGF-comparable levels in the control and glaucomatous retinas. The IOP. Both 22.5 kDa and 45 kDa VEGF-A 
165b densitometry readings in the retina following 10 days of elevated IOP. VEGF-A 
165b compared to the controls. Expression of the 22.5 kDa ratio of normalized VEGF-A 
165b glaucomatous retina.

**Figure 4.** Western blot analysis of VEGF-A 
165b isoforms, VEGF-A 
164 to the normal vitreous [20]. Among the anti-angiogenic VEGF-A isoforms remains unchanged compared to the normal vitreous [20]. For instance, the expression of the pro-angiogenic VEGF-A isoforms increases in the vitreous of human patients [20]. For example, VEGF-A 
165b inhibits neovascularization in the mouse retina following oxygen-induced retinopathy [21]. More recently, other inhibitory splice variants of VEGF-A have also been identified [19,20]. It has been suggested that the relative levels of the pro-angiogenic and anti-angiogenic VEGF-A isoforms determine whether angiogenesis will be stimulated or inhibited in a tissue [20]. For instance, the expression of the pro-angiogenic VEGF-A isoforms increases in the vitreous of human patients with diabetic retinopathy whereas the expression of the anti-angiogenic VEGF-A isoforms remains unchanged compared to the normal vitreous [20]. Among the anti-angiogenic VEGF-A isoforms, VEGF-A 
165b is observed to be the dominant isoform [15,18].

What molecular mechanism is responsible for the upregulation of VEGF-A 
165b mRNA in the glaucomatous retina? Although the precise answer remains unknown, proposed mechanisms include differential promoter selection, alternate regulation of mRNA stability, and regulation of alternative splicing [15,30-33]. In alternative splicing, as the VEGF-A gene is being transcribed, the emerging pre-mRNA is instantaneously processed by several RNA-binding proteins and splice factors [15]. These proteins bind to the auxiliary sequences on the pre-mRNA and determine which exons will be spliced [34]. It is thought that several signal transduction pathways, which are activated in response to changes in the environment (e.g., receptor-mediated pathways, neuronal activity, cellular stress-like hypoxia) affect alternative splicing by altering the relative levels of RNA-binding proteins and splice factors or the localization of splice factors...
within the cell [15,35,36]. More recently, microRNAs have also been shown to alter alternative splicing [37]. For example, in muscle and neuronal development, microRNAs lead to the inclusion of alternative exons by suppressing a repressor protein of alternative splicing [38,39]. In addition, transcriptional events may also affect the regulation of alternative splicing. For instance, the speed of RNA polymerase II can influence the choice of splice sites and recruitment of regulatory factors [40].

In conclusion, we report an increase in the retinal levels of the anti-angiogenic VEGF-A\textsubscript{165b} but not the pro-angiogenic VEGF-A\textsubscript{164} in our experimental glaucoma model. The combination of these findings suggests that the elevation of VEGF-A\textsubscript{165b} levels and/or the unchanged levels of VEGF-A\textsubscript{164} contribute to the lack of neovascularization in the retina in glaucoma.

**ACKNOWLEDGMENTS**

This work was financially supported by R01-EY13399 (C.L.G.), T32-EY07145 (C.E.), Massachusetts Lions Grant (C.L.G.), and MEEI Vision-Core Grant EY014104.

---

**REFERENCES**

1. Feke GT, Pasquale LR. Retinal Blood Flow Response to Posture Change in Glaucoma Patients Compared with Healthy Subjects. Ophthalmology 2008; 115:246-52. [PMID: 17689612]
2. Ulrich A, Ulrich C, Barth T, Ulrich WD. Detection of disturbed autoregulation of the peripapillary choroid in primary open angle glaucoma. Ophthalmic Surg Lasers 1996; 27:746-57. [PMID: 8878192]
3. Gugleta K, Orgul S, Hasler PW, Picornell T, Gherghel D, Flammer J. Choroidal vascular reaction to hand-grip stress in subjects with vasospasm and its relevance in glaucoma. Invest Ophthalmol Vis Sci 2003; 44:1573-80. [PMID: 12657594]
4. Fuchsjager-Mayrl G, Wally B, Georgopoulos M, Rainer G, Kircher K, Buehl W, Amoako-Mensah T, Eichler HG, Vass C, Schmetterer L. Ocular blood flow and systemic blood pressure in patients with primary open-angle glaucoma and ocular hypertension. Invest Ophthalmol Vis Sci 2004; 45:834-9. [PMID: 14985298]
5. Okuno T, Sugiyama T, Kojima S, Nakajima M, Ikeda T. Diurnal variation in microcirculation of ocular fundus and visual field change in normal-tension glaucoma. Eye 2004; 18:697-702. [PMID: 14739932]
6. Evans DW, Harris A, Garrett M, Chung HS, Kagemann L. Glaucoma patients demonstrate faulty autoregulation of
ocular blood flow during posture change. Br J Ophthalmol 1999; 83:809-13. [PMID: 10381668]
7. Galambos P, Vafiadis J, Vilchez SE, Wagenfeld L, Matthiasen ET, Richard G, Klemm M, Zeitz O. Compromised autoregulatory control of ocular hemodynamics in glaucoma patients after postural change. Ophthalmology 2006; 113:1832-6. [PMID: 16920194]
8. Grunwald JE, Riva CE, Stone RA, Keates EU, Petrig BL. Retinal autoregulation in open-angle glaucoma. Ophthalmology 1984; 91:1690-4. [PMID: 6521997]
9. Flammer J, Orgul S, Costa VP, Orzalesi N, Krieglstein GK, Serra LM, Renard JP, Stefansson E. The impact of ocular blood flow in glaucoma. Prog Retin Eye Res 2002; 21:359-93. [PMID: 12150988]
10. Ciolfi GA, Wang L. Optic nerve blood flow in glaucoma. Semin Ophthalmol 1999; 14:164-70. [PMID: 10790581]
11. Semenza GL. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J Appl Physiol 2000; 88:1474-80. [PMID: 10749844]
12. Roy H, Bhardwaj S, Yla-Herttuala S. Biology of vascular endothelial growth factors. FEBS Lett 2006; 580:2879-87. [PMID: 16631753]
13. Kwak N, Okamoto N, Wood JM, Campochiaro PA. VEGF is major stimulator in model of choroidal neovascularization. Invest Ophthalmol Vis Sci 2000; 41:3158-64. [PMID: 10967078]
14. Ozaki H, See MS, Ozaki K, Yamada H, Yamada E, Okamoto N, Hofmann F, Wood JM, Campochiaro PA. Blockade of vascular endothelial cell growth factor receptor signaling is sufficient to completely prevent retinal neovascularization. Am J Pathol 2000; 156:697-707. [PMID: 10666398]
15. Ladomery MR, Harper SJ, Bates DO. Alternative splicing in angiogenesis: the vascular endothelial growth factor paradigm. Cancer Lett 2007; 249:133-42. [PMID: 17627147]
16. Holmes DI, Zachary I. The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. Genome Biol 2005; 6:209. [PMID: 15693956]
17. Gerhardinger C, Brown LF, Roy S, Mizutani M, Zucker CL, Lorenzi M. Expression of vascular endothelial growth factor (VEGF) in the human retina and in nonproliferative diabetic retinopathy. Am J Pathol 1998; 152:1453-62. [PMID: 9626050]
18. Bates DO, Cui TG, Doughty JM, Winkler M, Sugiono M, Shields JD, Peat D, Gillatt D, Harper SJ. VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, is down-regulated in renal cell carcinoma. Cancer Res 2002; 62:4123-31. [PMID: 12124351]
19. Woolard J, Wang WY, Bevan HS, Qiuy, Morbidelli L, Pritchard-Jones RO, Cui TG, Sugiono M, Waine E, Perrin R, Foster R, Digby-Bell J, Shields JD, Whittles CE, Mushens RE, Gillatt DA, Ziche M, Harper SJ, Bates DO. VEGF165b, an inhibitory vascular endothelial growth factor splice variant: mechanism of action, in vivo effect on angiogenesis and endogenous protein expression. Cancer Res 2004; 64:7822-35. [PMID: 15520188]
20. Perrin RM, Konopatskaya O, Qiuy, Harper S, Bates DO, Churchill AJ. Diabetic retinopathy is associated with a switch in splicing from anti- to pro-angiogenic isoforms of vascular endothelial growth factor. Diabetologia 2005; 48:2422-7. [PMID: 16193288]
21. Konopatskaya O, Churchill AJ, Harper SJ, Bates DO, Gardiner TA. VEGF165b, an endogenous C-terminal splice variant of VEGF, inhibits retinal neovascularization in mice. Mol Vis 2006; 12:626-32. [PMID: 16735996]
22. Lip PL, Felmunden DC, Blann AD, Mathieu N, Thakur S, Cunliffe IA, Lip GY. Plasma vascular endothelial growth factor, soluble VEGF receptor FLT-1, and von Willebrand factor in glaucoma. Br J Ophthalmol 2002; 86:1299-302. [PMID: 12360933]
23. Hu DN, Ritch R, Liebmann J, Liu Y, Cheng B, Hu MS. Vascular endothelial growth factor is increased in aqueous humor of glaucomatous eyes. J Glaucoma 2002; 11:406-10. [PMID: 12362079]
24. Morrison JC, Moore CG, Deppe M, Gold BG, Meshul CK, Johnson EC. A rat model of chronic pressure-induced optic nerve damage. Exp Eye Res 1997; 64:85-96. [PMID: 9093024]
25. Moore CG, Milne ST, Morrison JC. Noninvasive measurement of rat intraocular pressure with the Tono-Pen. Invest Ophthalmol Vis Sci 1993; 34:363-9. [PMID: 8440590]
26. Yang G, Cai KQ, Thompson-Lanza JA, Cast RC Jr, Liu J. Inhibition of breast and ovarian tumor growth through multiple signaling pathways by using retrovirus-mediated small interfering RNA against Her-2/neu gene expression. J Biol Chem 2004; 279:4339-45. [PMID: 14625284]
27. Mor F, Quintana FJ, Cohen IR. Angiogenesis-inflammation cross-talk: vascular endothelial growth factor is secreted by activated T cells and induces Th1 polarization. J Immunol 2004; 172:4618-23. [PMID: 15034080]
28. Kim I, Ryan AM, Rohan R, Amano S, Aguilar S, Miller JW, Adams AM. Constitutive expression of VEGF, VEGFR-1, and VEGFR-2 in normal eyes. Invest Ophthalmol Vis Sci 1999; 40:2115-21. [PMID: 10440268]
29. Ozaki H, Yu, Della N, Ozaki K, Luna JD, Yamada H, Hackett SF, Okamoto N, Zack DJ, Semenza GL, Campochiaro PA. Hypoxia inducible factor-1alpha is increased in ischemic retina: temporal and spatial correlation with VEGF expression. Invest Ophthalmol Vis Sci 1999; 40:182-9. [PMID: 9888442]
30. Bates DO, MacMillan PP, Manjalgy, Qiuy, Hudson SJ, Bevan HS, Hunter AJ, Soothill PW, Read M, Donaldson LF, Harper SJ. The endogenous anti-angiogenic family of splice variants of VEGF, VEGFxxxb, are down-regulated in pre-eclamptic placentae at term. Clin Sci (Lond) 2006; 110:575-85. [PMID: 16451124]
31. Huez I, Bornes S, Bresson D, Creancier L, Prats H. New vascular endothelial growth factor isoform is expressed in internal ribosome entry site-driven CUG translation initiation. Mol Endocrinol 2001; 15:2197-210. [PMID: 11731620]
32. Levy NS, Goldberg MA, Levy AP. Sequencing of the human vascular endothelial growth factor (VEGF) 3′ untranslated region (UTR): conservation of five hypoxia-inducible RNA-protein binding sites. Biochim Biophys Acta 1997; 1352:167-73. [PMID: 9199248]
33. Cui TG, Foster RR, Saleem M, Mathieson PW, Gillatt DA, Bates DO, Harper SJ. Differentiated human podocytes endogenously express an inhibitory isoform of vascular endothelial growth factor (VEGF165b) mRNA and protein. Am J Physiol Renal Physiol 2004; 286:F767-73. [PMID: 14644752]
34. Matlin AJ, Clark F, Smith CW. Understanding alternative splicing: towards a cellular code. Nat Rev Mol Cell Biol 2005; 6:386-98. [PMID: 15956978]

35. Stamm S. Signals and their transduction pathways regulating alternative splicing: a new dimension of the human genome. Hum Mol Genet 2002; 11:2409-16. [PMID: 12351576]

36. Hagiwara M. Alternative splicing: a new drug target of the post-genome era. Biochim Biophys Acta 2005; 1754:324-31. [PMID: 16260193]

37. Makeyev EV, Maniatis T. Multilevel regulation of gene expression by microRNAs. Science 2008; 319:1789-90. [PMID: 18369137]

38. Boutz PL, Chawla G, Stoilov P, Black DL. MicroRNAs regulate the expression of the alternative splicing factor nPTB during muscle development. Genes Dev 2007; 21:71-84. [PMID: 17210790]

39. Makeyev EV, Zhang J, Carrasco MA, Maniatis T. The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Mol Cell 2007; 27:435-48. [PMID: 17679093]

40. Kornblihtt AR. Chromatin, transcript elongation and alternative splicing. Nat Struct Mol Biol 2006; 13:5-7. [PMID: 16395314]