Molecular Vision **1999; 5:34** <http://www.molvis.org/molvis/v5/p34>
Received 24 May 1999 | Accepted 2 Nov 1999 | Published 3 Nov 1999

The pathogenesis of choroidal neovascularization in patients with age-related macular degeneration

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Laser photocoagulation and several experimental treatments for choroidal neovascularization (CNV) in patients with age-related macular degeneration attempt to ablate the neovascularization, but do not address underlying angiogenic stimuli. As a result, recurrences are a major problem. Drug treatment to counter the growth of CNV would be a major advance, but its development is impeded by lack of knowledge concerning the stimuli and other molecular signals involved in the pathogenesis of CNV. Herein we explore clues that can be gleaned from clinical, epidemiological, pathological, and experimental data. These suggest that abnormalities of the extracellular matrix of retinal pigmented epithelial (RPE) cells may promote a pro-angiogenic RPE phenotype that contributes to the development of CNV. This provides a general hypothesis that can be tested, but it is also necessary to test hypotheses regarding the specific alterations in gene expression that contribute to CNV. Identification of alterations in gene expression will provide targets for rational design of drug treatment.

Choroidal neovascularization (CNV) is the major cause of severe vision loss in patients with age-related macular degeneration (AMD)[1]. The neovascularization originates from choroidal blood vessels and grows through Bruch's membrane, usually at multiple sites, into the sub-retinal pigmented epithelial (RPE) space [2,3]. It may result in accumulation of serum and/or blood beneath the RPE, a situation referred to as pigment epithelial detachment (PED). The RPE usually becomes incompetent, causing serous retinal detachment. Often the neovascularization extends through the RPE into the subretinal space resulting in increased fluid and often blood in the subretinal space. Collection of fluid and/or blood in the subretinal space makes the surface of the retina irregular such that straight lines appear crooked to the patient, a condition referred to as metamorphopsia. This is the most common presenting complaint of patients with CNV. Fluorescein angiography is used to identify and localize the CNV, but usually some or all of the CNV is obscured by RPE cells that proliferate and partially or completely surround the new vessels. When CNV cannot be well-localized by fluorescein angiography, it is referred to as occult. Often there is a component of occult CNV along with well-delineated or classic CNV. If classic CNV is present, the visual prognosis is worse than if there is only occult CNV [4], suggesting that the response of the RPE that obscures blood vessels may also favorably modify the clinical course. Over time there is growth of the new vessels and accompanying proliferation of the RPE. Subretinal hemorrhage stimulates this scarring process, but scarring also occurs in its absence, and results in the death of overlying photoreceptors and a permanent central scotoma [2].

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If the new vessels of AMD patients presenting with CNV can be localized with fluorescein angiography and do not appear to involve the fovea (a rare combination), an attempt is made to eliminate the CNV with laser photocoagulation. The Macular Photocoagulation Study demonstrated that patients treated with laser photocoagulation are less likely to experience severe visual loss than untreated patients, but initial benefits are lost in the majority of patients due to recurrent CNV [1]. When the CNV involves the center of the fovea, laser photocoagulation can be considered to ablate the new vessels and prevent further growth, thereby limiting the size of the scar, but the fovea is destroyed, and reading vision is permanently lost [5]. Several experimental approaches that destroy subfoveal CNV while sparing the fovea are being tested, including surgical removal of CNV [6], macular translocation [7], and photodynamic therapy [8]. None of these approaches deal with the underlying stimuli for neovascularization, and for each, recurrences are a problem. Drug treatments that block stimuli for CNV growth would be a major advance, but development of such treatment is hindered by our poor understanding of the pathogenesis of CNV. This article discusses clues that can be used to generate testable hypotheses concerning the pathogenesis of CNV and data that are currently available.

What can we extrapolate from neovascularization elsewhere in the body? Angiogenesis is a critical process during embryonic development and wound repair and occurs in almost all tissues of the body. However, if the nature or extent of the wound (insult) is such that stimuli are excessive and/or inhibitory influences are compromised, then pathologies such as CNV may result. Angiogenesis has several key steps, many of which CNV is likely to share with neovascularization elsewhere in the body, but the molecular signals involved in each of these steps can vary and must be determined for the tissue of interest. Endothelial cells differ in different parts of the body,

and surrounding cells participate in the neovascular response resulting in tissue-specific aspects. As noted above, RPE cells play a major role in CNV, but not in any other type of neovascularization.

One key step in angiogenesis is alteration in the balance between pro-angiogenic and anti-angiogenic factors. Soluble factors that have been demonstrated to stimulate angiogenesis in some systems include vascular endothelial growth factor (VEGF) [9], members of the fibroblast growth factor (FGF) family [10], tumor necrosis factor-α(TNF-α) [11], insulin-like growth factor I (IGF-I) [12,13], hepatocyte growth factor (HGF) [14], and others. Members of the transforming growth factor-β (TGF-β) family have been shown to have anti-angiogenic effects in some systems and inhibit endothelial cell migration and repair after injury [15]. It is not known which if any of these factors are involved in CNV.

Along with soluble pro-angiogenic and anti-angiogenic factors, extracellular matrix (ECM) molecules also participate in several ways in the regulation of new blood vessel growth. ECM molecules may bind and sequester soluble factors, preventing them from activating receptors on endothelial cells until they are released from the ECM by proteolysis [16-18]. ECM molecules may directly stimulate or inhibit endothelial cell processes involved in angiogenesis by binding to integrins, cell surface heterodimer receptors that when activated can upregulate or downregulate various intracellular signaling pathways [19,20]. Remodeling of the ECM may alter integrin-mediated signaling in some types of endothelial cells and promote angiogenesis. On the other hand, pro-angiogenic factors may act in part by altering expression of integrins on endothelial cells [21]. Endothelial cells of dermal vessels have increased expression of αvβ3 integrin when participating in angiogenesis and αvβ3 antagonists block angiogenesis [22].

Another general principle is that pro-angiogenic factors stimulate processes in endothelial cells, including proteolytic activity, migration, proliferation, and tube formation [23,24]. Two proteolytic systems have been implicated in the breakdown of ECM during angiogenesis, one involving the urokinase type of plasminogen activator (uPA) [25] and one involving matrix metalloproteinases (MMPs) [26,27]. Are these systems both important in the development of CNV or is one relatively more important? The answer to this question has therapeutic implications, because both systems are modulated by endogenous and pharmacological inhibitors that could potentially be exploited in the design of treatments for CNV.

What can be extrapolated from angiogenesis elsewhere in the eye? Compared to CNV, much more is known about neovascularization in the retina, which has a similar but not identical microenvironment to the choroid. Numerous clinical and experimental observations have demonstrated the central role of hypoxia or ischemia in the development of retinal neovascularization [28-30]. Occlusion of retinal vessels leading to retinal ischemia is a feature shared by each of the disease processes in which retinal neovascularization occurs and hence they are referred to as ischemic retinopathies. Does hypoxia play a role in the development of CNV? The next article in this series will discuss this possibility. Briefly, there is some suggestion that choroidal blood flow may be altered in patients with AMD [31,32], but it is not clear if this is sufficient to cause hypoxia. Another possible source of hypoxia that has been suggested is diffuse thickening of Bruch's membrane with lipophilic material decreasing diffusion of oxygen from the choroid to the RPE and retina, but there are no data to support this hypothesis. Also, hypoxia is unlikely to be present in other disease processes associated with CNV that occur in young patients, such as ocular histoplasmosis.

Vascular endothelial growth factor (VEGF) is a major stimulatory factor for retinal neovascularization. It is unlikely to be the only stimulatory factor, because insulin-like growth factor I may also participate [13], but there is strong evidence indicating that VEGF plays a central role. It is upregulated by hypoxia [33,34] and its levels are increased in the retina and vitreous of patients [35-38] or laboratory animals [39,40] with ischemic retinopathies. Increased expression of VEGF in retinal photoreceptors of rhodopsin/VEGF transgenic mice stimulates neovascularization within the retina [41,42] and VEGF antagonists partially inhibit retinal or iris NV in animal models [43-45].

Is VEGF a stimulatory factor for CNV? There is circumstantial evidence suggesting that VEGF may be involved, because it is present in fibroblastic cells and transdifferentiated RPE cells of surgically removed choroidal neovascular membranes [46-48]. Also, in both rat and monkey models of laser-induced choroidal neovascularization, increases in VEGF mRNA are seen in RPE-like cells, choroidal vascular endothelial cells, and fibroblast-like cells in the lesions [49-51]. However, increased expression of VEGF in photoreceptors does not result in CNV [41]. Therefore, additional studies are needed to determine if VEGF is involved in the development of CNV.

Compared to VEGF, there is much less evidence implicating FGF2 in the development of retinal neovascularization. Recently, using both FGF2 knockout mice and transgenic mice that overexpress FGF2 in photoreceptors, Ozaki and associates demonstrated that FGF2 is neither necessary nor sufficient for the development of retinal neovascularization [52]. Similarly, increased expression of FGF2 is neither necessary nor sufficient for the development of CNV [53]. This does not mean that FGF2 plays no role in the pathogenesis of CNV, but if it does play a role, there must be something more than just increased expression of FGF2 involved. Perhaps in the setting in which CNV occurs, there are conditions that increase the amount of FGF2 in the extracellular space. This would be consistent with studies demonstrating that sustained release of FGF2 in the subretinal space of minipigs [54] or rabbits [55] results in choroidal neovascularization.

What is the setting in which CNV occurs in AMD? Clinical risk factors for the development of CNV in patients with AMD are large confluent drusen, pigmentary changes, and choroidal neovascularization in the fellow eye [56]. Smoking significantly increases the risk of CNV [57]. The strongest pathological association with CNV is diffuse sub-RPE deposits [2].

Drusen and thickening of Bruch's membrane are abnormalities of the ECM of the RPE. Pigmentary changes are usually due to drop out and/or proliferation of RPE cells, and

pathologic studies confirm that these occur in patients with AMD [58]. Smoking could contribute by exacerbating vascular disease, but it is also a strong oxidative stress and could worsen oxidative damage to the retina and RPE. Oxidative stress results in deposition of ECM along Bruch's membrane [59] and increased production of FGF2 in RPE cells [60]. Mousa and associates have demonstrated that exposure of cultured RPE cells to certain ECM molecules, most notably thrombospondin-1, increases VEGF and to a lesser extent FGF2 in cell supernatants [61]. This suggests that alteration of integrin signaling may influence externalization of VEGF and FGF2 from RPE cells. Taken together, these epidemiological, pathological, and experimental data suggest that alteration of the ECM of RPE cells may play an important role in the development of CNV in patients with AMD.

What are other diseases in which choroidal neovascularization occurs and what do they have in common with AMD? Diseases that lead to abnormalties or breaks in Bruch's membrane, such as Pseudoxanthoma Elasticum or high myopia, in which Bruch's membrane is thinned, have an increased risk of CNV [62]. Mechanical (trauma) or thermal (laser) damage to Bruch's membrane are also associated with increased risk of CNV. A third category of increased risk is inflammatory diseases of the choroid such as multifocal choroiditis or ocular histoplasmosis. Finally, there is Sorsby's Fundus Dystrophy, an autosomal dominant inherited disease in which patients have deposits along Bruch's membrane and high incidences of CNV [63,64]. Some patients with Sorsby's have mutations in the tissue inhibitor of metalloproteinases 3 (TIMP-3) gene, which has a product that is involved in regulation of ECM turnover [65]. Therefore, these diseases and AMD all exhibit abnormalities in or around Bruch's membrane suggesting that alteration of the ECM of the RPE predisposes the development of CNV.

What can we learn about the pathogenesis of CNV from animal models? Rupture of Bruch's membrane with laser photocoagulation is a reliable way to produce CNV and has been used to establish models in primates [66], rabbits [67], rats [68,69], and mice [53]. Sustained release of FGF2 in minipigs [54] or rabbits [55] and sustained release of VEGF in primates [70] also causes CNV. However, as noted above, increased expression of VEGF or FGF2 in photoreceptors of transgenic mice does not result in CNV [41,52]. Perhaps surgical trauma to Bruch's membrane, resulting in disturbed ECM of the RPE and/or perturbation of an endogenous inhibitor such as TIMP-3, is a critical component of sustained release models that is absent from overexpression models. Additional studies are needed to determine if Bruch's membrane provides a physical and/or biochemical barrier to CNV. Demonstration of the molecular nature of any biochemical barrier would provide an important target for therapeutic intervention.

CONCLUSIONS

CNV is the major cause of severe loss of vision in patients with AMD. Treatments directed at ablating the new vessels are plagued by recurrences. Development of drug treatments that counteract the stimuli for new blood vessel growth are hindered by poor understanding of the pathogenesis of CNV. However, recent progress provides a framework for generation of experimental questions that could provide important new insights. A reasonable working hypothesis is that abnormalities of ECM in or around Bruch's membrane result in altered gene expression in RPE cells which favors development of CNV. New experimental techniques that allow manipulation of gene expression in the retina, RPE, or choroid should make it possible to answer experimental questions derived from this hypothesis and provide the knowledge necessary for rational design of drug treatment.

ACKNOWLEDGEMENTS

Supported by a grant from Odette Wurzburger, PHS grant EY05951, and core grant P30EY1765 from the National Eye Institute, a Lew R. Wasserman Merit Award (PAC) from Research to Prevent Blindness, Inc. (RPB), a Manpower Award (JWM) from RPB, an unrestricted grant from RPB, the Rebecca P. Moon, Charles M. Moon, Jr., and Dr. P. Thomas Manchester Research Fund, a grant from Mrs. Harry J. Duffey, a grant from Dr. and Mrs. William Lake, and a grant from Project Insight. PAC is the George S. and Dolores D. Eccles Professor of Ophthalmology.

REFERENCES

1. Argon laser photocoagulation for neovascular maculopathy. Five-year results from randomized clinical trials. Macular Photocaoagulation Study Group. Arch Ophthalmol 1991; 109:1109-14.
2. Green WR, Enger C. Age-related macular degeneration histopathologic studies. The 1992 Lorenz E. Zimmerman Lecture. Ophthalmology 1993; 100:1519-35.
3. Chang TS, Freund KB, de la Cruz Z, Yannuzzi LA, Green WR. Clinicopathologic correlation of choroidal neovascularization demonstrated by indocyanine green angiography in a patient with retention of good vision for almost four years. Retina 1994; 14:114-24.
4. Occult choroidal neovascularization. Influence on visual outcome in patients with age-related macular degeneration. Macular Photocoagulation Study Group. Arch Ophthalmol 1996; 114:400-12.
5. Visual outcome after laser photocoagulation for subfoveal choroidal neovascularization secondary to age-related macular degeneration. The influence of initial lesion size and initial visual acuity. Macular Photocoagulation Study Group. Arch Ophthalmol 1994; 112:480-8.
6. Thomas MA, Dickinson JD, Melberg NS, Ibanez HE, Dhaliwal RS. Visual results after surgical removal of subfoveal neovascular membranes. Ophthalmology 1994; 101:1384-96.
7. de Juan E Jr, Loewenstein A, Bressler NM, Alexander J. Translocation of the retina for management of subfoveal choroidal neovascularization II: a preliminary report in humans. Am J Ophthalmol 1998; 125:635-46.
8. Husain D, Miller JW, Michaud N, Connolly E, Flotte TJ, Gragoudas ES. Intravenous infusion of liposomal benzoporphyrin derivative for photodynamic therapy of experimental choroidal neovascularization. Arch Ophthalmol 1996; 114:978-85.
9. Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley BL, Delfino JJ, Siegel NR, Leimgruber RM, Feder J. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 1989; 84:1470-8.
10. Abraham JA, Mergia A, Whang JL, Tumolo A, Friedman J, Hjerrild KA, Gospodarowicz D, Fiddes JC. Nucleotide sequence of a bovine clone encoding the angiogenic protein, basic fibro-

blast growth factor. Science 1986; 233:545-8.
11. Leibovich SJ, Polverini PJ, Shepard HM, Wiseman DM, Shively V, Nuseir N. Macrophage-induced angiogenesis is mediated by tumour necrosis factor-alpha. Nature 1987; 329:630-2.
12. Grant MB, Mames RN, Fitzgerald C, Ellis EA, Caballero S, Chegini N, Guy J. Insulin-like growth factor I as an angiogenic agent. In vivo and in vitro studies. Ann N Y Acad Sci 1993; 692:230-42.
13. Smith LE, Kopchick JJ, Chen W, Knapp J, Kinose F, Daley D, Foley E, Smith RG, Schaeffer JM. Essential role of growth hormone in ischemia-induced retinal neovascularization. Science 1997; 276:1706-9.
14. Laterra J, Nam M, Rosen E, Rao JS, Lamszus K, Goldberg ID, Johnston P. Scatter factor/hepatocyte growth factor gene transfer enhances glioma growth and angiogenesis in vivo. Lab Invest 1997; 76:565-77.
15. Madri JA, Reidy MA, Kocher O, Bell L. Endothelial cell behavior after denudation injury is modulated by transforming growth factor-beta1 and fibronectin. Lab Invest 1989; 60:755-65.
16. Vlodavsky I, Folkman J, Sullivan R, Fridman R, Ishai-Michaeli R, Sasse J, Klagsbrun M. Endothelial cell-derived basic fibroblast growth factor: synthesis and deposition into subendothelial extracellular matrix. Proc Natl Acad Sci U S A 1987; 84:2292-6.
17. Vlodavsky I, Korner G, Ishai-Michaeli R, Bashkin P, Bar-Shavit R, Fuks Z. Extracellular matrix-resident growth factors and enzymes: possible involvement in tumor metastasis and angiogenesis. Cancer Metastasis Rev 1990; 9:203-26.
18. Park JE, Keller GA, Ferrara N. The vascular endothelial growth factor (VEGF) isoforms: differential deposition into the subepithelial extracellular matrix and bioactivity of extracellular matrix-bound VEGF. Mol Biol Cell 1993; 4:1317-26.
19. Dike LE, Ingber DE. Integrin-dependent induction of early growth response genes in capillary endothelial cells. J Cell Sci 1996; 109:2855-63.
20. Schwartz MA, Ingber DE. Integrating with integrins. Mol Biol Cell 1994; 5:389-93.
21. Friedlander M, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, Cheresh DA. Definition of two angiogenic pathways by distinct alpha v integrins. Science 1995; 270:1500-2.
22. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science 1994; 264:569-71.
23. Gross JL, Moscatelli D, Rifkin DB. Increased capillary endothelial cell protease activity in response to angiogenic stimuli in vitro. Proc Natl Acad Sci U S A 1983; 80:2623-7.
24. Sato Y, Rifkin DB. Autocrine activities of basic fibroblast growth factor: regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. J Cell Biol 1988; 107:1199-205.
25. Pepper MS, Vassalli JD, Montesano R, Orci L. Urokinase-type plasminogen activator is induced in migrating capillary endothelial cells. J Cell Biol 1987; 105:2535-41.
26. Moscatelli DA, Rifkin DB, Jaffe EA. Production of latent collagenase by human umbilical vein endothelial cells in response to angiogenic preparations. Exp Cell Res 1985; 156:379-90.
27. Cornelius LA, Nehring LC, Roby JD, Parks WC, Welgus HG. Human dermal microvascular endothelial cells produce matrix metalloproteinases in response to angiogenic factors and migration. J Invest Dermatol 1995; 105:170-6.
28. Michaelson I. The mode of development of the vascular system of the retina with some observations on its significance for certain retinal diseases. Trans Ophthalmol Soc U K 1948; 68:137-80.
29. Ashton N. Retinal vascularization in health and disease. Am J Ophthalmol 1957; 44:7-17.
30. Shimizu K, Kobayashi Y, Muraoka K. Midperipheral fundus involvement in diabetic retinopathy. Ophthalmology 1981; 88:601-12.
31. Grunwald JE, Hariprasad SM, DuPont J, Maguire MG, Fine SL, Brucker AJ, Maguire AM, Ho AC. Foveolar choroidal blood flow in age-related macular degeneration. Invest Ophthalmol Vis Sci 1998; 39:385-90.
32. Ross RD, Barofsky JM, Cohen G, Baber WB, Palao SW, Gitter KA. Presumed macular choroidal watershed vascular filling, choroidal neovascularization, and systemic vascular disease in patients with age-related macular degeneration. Am J Ophthalmol 1998; 125:71-80.
33. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 1992; 359:843-5.
34. Plate KH, Breier G, Weich HA, Risau W. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature 1992; 359:845-8.
35. Adamis AP, Miller JW, Bernal MT, D'Amico DJ, Folkman J, Yeo TK, Yeo KT. Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. Am J Ophthalmol 1994; 118:445-50.
36. Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 1994; 331:1480-7.
37. Malecaze F, Clamens S, Simorre-Pinatel V, Mathis A, Chollet P, Favard C, Bayard F, Plouet J. Detection of vascular endothelial growth factor messenger RNA and vascular endothelial growth factor-like activity in proliferative diabetic retinopathy. Arch Ophthalmol 1994; 112:1476-82.
38. Pe'er J, Shweiki D, Itin A, Hemo I, Gnessin H, Keshet E. Hypoxia-induced expression of vascular endothelial growth factor by retinal cells is a common factor in neovascularizing ocular diseases. Lab Invest 1995; 72:638-45.
39. Miller JW, Adamis AP, Shima DT, D'Amore PA, Moulton RS, O'Reilly MS, Folkman J, Dvorak HF, Brown LF, Berse B, et al. Vascular endothelial growth factor/vascular permeability factor is temporally and spatially correlated with ocular angiogenesis in a primate model. Am J Pathol 1994; 145:574-84.
40. Pierce EA, Avery RL, Foley ED, Aiello LP, Smith LE. Vascular endothelial growth factor/vascular permeability factor expression in a mouse model of retinal neovascularization. Proc Natl Acad Sci U S A 1995; 92:905-9.
41. Okamoto N, Tobe T, Hackett SF, Ozaki H, Vinores MA, LaRochelle W, Zack DJ, Campochiaro PA. Transgenic mice with increased expression of vascular endothelial growth factor in the retina: a new model of intraretinal and subretinal neovascularization. Am J Pathol 1997; 151:281-91.
42. Tobe T, Okamoto N, Vinores MA, Derevjanik NL, Vinores SA, Zack DJ, Campochiaro PA. Evolution of neovascularization in mice with overexpression of vascular endothelial growth factor in photoreceptors. Invest Ophthalmol Vis Sci 1998; 39:180-8.
43. Aiello LP, Pierce EA, Foley ED, Takagi H, Chen H, Riddle L, Ferrara N, King GL, Smith LE. Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. Proc Natl Acad Sci U S A 1995; 92:10457-61.
44. Robinson GS, Pierce EA, Rook SL, Foley E, Webb R, Smith LE. Oligodeoxynucleotides inhibit retinal neovascularization in a murine model of proliferative retinopathy. Proc Natl Acad Sci U S A 1996; 93:4851-6.

45. Adamis AP, Shima DT, Tolentino MJ, Gragoudas ES, Ferrara N, Folkman J, D'Amore PA, Miller JW. Inhibition of vascular endothelial growth factor prevents retinal ischemia-associated iris neovascularization in a nonhuman primate. Arch Ophthalmol 1996; 114:66-71.
46. Frank RN, Amin RH, Eliott D, Puklin JE, Abrams GW. Basic fibroblast growth factor and vascular endothelial growth factor are present in epiretinal and choroidal neovascular membranes. Am J Ophthalmol 1996; 122:393-403.
47. Kvanta A, Algvere PV, Berglin L, Seregard S. Subfoveal fibrovascular membranes in age-related macular degeneration express vascular endothelial growth factor. Invest Ophthalmol Vis Sci 1996; 37:1929-34.
48. Lopez PF, Sippy BD, Lambert HM, Thach AB, Hinton DR. Transdifferentiated retinal pigment epithelial cells are immunoreactive for vascular endothelial growth factor in surgically excised age-related macular degeneration-related choroidal neovascular membranes. Invest Ophthalmol Vis Sci 1996; 37:855-68.
49. Ogata N, Matsushima M, Takada Y, Tobe T, Takahashi K, Yi X, Yamamoto C, Yamada H, Uyama M. Expression of basic fibroblast growth factor mRNA in developing choroidal neovascularization. Curr Eye Res 1996; 15:1008-18.
50. Yi X, Ogata N, Komada M, Yamamoto C, Takahashi K, Omori K, Uyama M. Vascular endothelial growth factor expression in choroidal neovascularization in rats. Graefes Arch Clin Exp Ophthalmol 1997; 235:313-9.
51. Husain D, Ryan AM, Cuthbertson RA, Gragoudas ES, Adamis AP, Michaud N Miller JW. Vascular encothelial growth factor (VEGF) expression is correlated with choroidal neovascularization in a monkey model. Invest Ophthalmol Vis Sci 1997; 38:S501.
52. Ozaki H, Okamoto N, Ortega S, Chang M, Ozaki K, Sadda S, Vinores MA, Derevjanik N, Zack DJ, Basilico C, Campochiaro PA. Basic fibroblast growth factor in neither necessary nor sufficient for the development of retinal neovascularization. Am J Pathol 1998; 153:757-65.
53. Tobe T, Ortega S, Luna LD, Ozaki H, Okamoto N, Derevjanik NL, Vinores SA, Basilico C, Campochiaro PA. Targeted disruption of the FGF2 gene does not prevent choroidal neovascularization in a murine model. Am J Pathol 1998; 153:1641-6.
54. Soubrane G, Cohen SY, Delayre T, Tassin J, Hartmann MP, Coscas GJ, Courtois Y, Jeanny JC. Basic fibroblast growth factor experimentally induced choroidal angiogenesis in the minipig. Curr Eye Res 1994; 13:183-95.
55. Kimura H, Sakamoto T, Hinton DR, pee C, Ogura Y, Tabata Y, Ikada Y, Ryan SJ. A new model of subretinal neovascularization in the rabbit. Invest Ophthalmol Vis Sci 1995; 36:2110-9.
56. Bressler SB, Maguire MG, Bressler NM, Fine SL. Relationship of drusen and abnormalities of the retinal pigment epithelium to the prognosis of neovascular macular degeneration. The Macular Photocoagulation Study Group. Arch Ophthalmol 1990; 108:1442-7.
57. Klein R, Klein BE, Linton KL, DeMets DL. The Beaver Dam Eye Study: the relation of age-related maculopathy to smoking. Am J Epidemiol 1993; 137:190-200.
58. Bressler NM, Silva JC, Bressler SB, Fine SL, Green WR. Clinicopathologic correlation of drusen and retinal pigment epithelial abnormalities in age-related macular degeneration. Retina 1994; 14:130-42.
59. Gottsch JD, Bynoe LA, Harlan JB, Rencs EV, Green WR. Light-induced deposits in Bruch's membrane of protoporphyric mice. Arch Ophthalmol 1993; 111:126-9.
60. Hackett SF, Schoenfeld CL, Freund J, Gottsch JD, Bhargave S, Campochiaro PA. Neurotrophic factors, cytokines and stress increase expression of basic fibroblast growth factor in retinal pigmented epithelial cells. Exp Eye Res 1997; 64:865-73.
61. Mousa SA, Lorelli W, Campochiaro PA. Role of hypoxia and extracellular matrix-integrin binding in the modulation of angiogenic growth factors secretion by retinal pigmented epithelial cells. J Cell Biochem 1999; 74:135-43.
62. Green WR, Wilson DJ. Choroidal neovascularization. Ophthalmology 1986; 93:1169-76.
63. Sorsby A, Mason MEJ, Gardener N. A fundus dystrophy with unusual features. Br J Ophthalmol 1949; 33:67-97.
64. Hoskin A, Sehmi K, Bird AC. Sorsby's pseudoinflammatory macular dystrophy. Br J Ophthalmol 1981; 65:859-65.
65. Weber BH, Vogt G, Pruett RC, Stohr H, Felbor U. Mutations in the tissue inhibitor of metalloproteinases-3 (TIMP3) in patients with Sorsby's fundus dystrophy. Nat Genet 1994; 8:352-6.
66. Ryan SJ. Subretinal neovascularization. Natural history of an experimental model. Arch Ophthalmol 1982; 100:1804-9.
67. elDirini AA, Ogden TE, Ryan SJ. Subretinal endophotocoagulation. A new model of subretinal neovascularization in the rabbit. Retina 1991; 11:244-9.
68. Frank RN, Das A, Weber ML. A model of subretinal neovascularization in the pigmented rat. Curr Eye Res 1989; 8:239-47.
69. Dobi ET, Puliafito CA, Destro M. A new model of experimental choroidal neovascularization in the rat. Arch Ophthalmol 1989; 107:264-9.
70. Cui JZ, Kimura H, Spee C, Murata T, Hinton DR, Ryan SJ. Time course of VEGF-induced subretinal neovascularization in the primate. Invest Ophthalmol Vis Sci 1997; 38:S357.