The Role of Vascular Endothelial Growth Factor in Neurogenesis in Adult Brain

Veronica Galvan, David A. Greenberg and Kunlin Jin*

Buck Institute for Age Research, Novato, CA 94945, USA

Abstract: VEGF is a canonical angiogenic factor. In addition, its role as a stimulator of neurogenesis was recently uncovered. Vascular and nervous networks share common molecular mechanisms underlying their morphogenesis. VEGF is likely to regulate both processes during development and in adult organisms.

Keywords: VEGF, VEGF receptor, neural stem cells, brain, neurogenesis, angiogenesis, growth factor, endothelial cells.

Until recently, a central belief in the field of neuroscience had been that new neurons do not arise in the adult mammalian brain. A few years ago, however, studies firmly established that neurogenesis takes place throughout adult life [1-11]. It is now widely accepted that undifferentiated neural stem/progenitor cells (NPCs) are maintained in specialized microenvironments in some brain regions [12-15]. In rodents and primates, these spontaneously neurogenic brain regions are the subventricular (SVZ) and the subependymal zone, and the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus [1, 6, 8, 9, 16]. That vessels form de novo in adult organisms, on the other hand, is a fact that has been known for centuries [17]. A canonical regulator of the process of vasculogenesis or angiogenesis, both in physiological conditions and as a consequence of injury is vascular endothelial growth factor (VEGF)[18, 19]. VEGF stimulates the division and migration of endothelial cells (ECs), is strongly induced by hypoxia [20-23], and couples hypoxia to angiogenesis in diverse tissues including the brain [24, 25].

Vascular and nervous networks show similar, parallel patterns of arborization during their 'choreographed' morphogenesis [26-29]. Recent discoveries suggest that these anatomical similarities may arise from shared molecular mechanisms and the notion of 'mutual guidance' has been proposed to explain the alignment of nerves and vessels in the adult [29]. While VEGF is critical for the guidance of ECs to their targets during development [30], several families of classical axon guidance molecules such as semaphorins [31, 32], ephrins [33, 34] and netrins [35, 36] have been shown to mediate vascular guidance effects as well. That the converse may be true is suggested by the ability of neuropilins to function simultaneously as semaphorin and VEGF receptors [37-39] (Fig. **1**), and by a role of VEGF in determinating both cell fate in the developing retina [40] and neuronal patterning in the facial nerve [41]. *In vitro*, VEGF acts as a chemoattractant for neuronal progenitors [42], increases axonal outgrowth [43], improves neuronal survival [44] and protects cells against ischemic [45], serum withdrawal-induced [46] and glutamate-mediated injury [47]. A direct effect of VEGF in the stimulation of proliferation of neuronal precursors was demonstrated by Zhu *et al*. *in vitro* [48]. Suggesting that the association between vasculogenesis and neurogenesis extends into the adult stage, VEGF stimulates spontaneous neurogenesis in the adult songbird[49] and rodent brain [50]. NPCs exist preferentially in close association with proliferating vascular elements in the DG and in the SVZ [49, 51], where dividing neuronal and endothelial precursor cells are contiguous [51]. Moreover, those ECs that are enriched in the environment surrounding clusters of NCs regulate their proliferation, and secrete factors that favor adoption of a neuronal fate *in vitro* [51].

Synaptogenesis [52] and neurogenesis [53] in neurogenic areas of the brain are strongly induced by experience such as enriched housing, learning/training and voluntary exercise. These behavioral interventions induce the expression of trophic factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), fibroblast growth factor-2 (FGF-2) and insulin-like growth factor-I (IGF-1) [54-58]. Likewise, VEGF expression is strongly induced by and mediates the neurogenic effects of running [59]. During *et al*. demonstrated that environmental enrichment and training in the Morris water maze also induce VEGF expression in rat hippocampus. Moreover, enforced expression of VEGF is sufficient to increase neurogenesis in the SGZ and enhance performance in tests of associative and spatial learning that depend on hippocampal function [60]. This could be attributed to VEGF's neurogenic, but not angiogenic, activity, as expression of placental growth factor (PGF) (a VEGF family member that interacts with VEGFR1 but not with VEGFR2 and that, like VEGF, stimulates angiogenesis) has negative effects on neurogenesis and inhibited learning, although it similarly stimulates angiogenesis. In addition, downregulation of VEGF expression using RNA interference abolishes enrichment-induced NPC proliferation, pointing out to a causal relationship between VEGF action and experience-stimulated neurogenesis.

Following stroke, VEGF promotes angiogenesis in the penumbra of the ischemic lesion [60] and reduces infarct size [61], possibly through a reduction in cell injury during the acute phase. The effect of VEGF after ischemia likely involves its neurogenic properties, as VEGF treatment

*Address correspondence to this author at the Buck Institute for Age Research, 8001 Redwood Blvd., Novato, CA94945-0638, USA; Tel: (415) 209-2086; Fax: (415) 209-2230; E-mail: kjin@buckcenter.org

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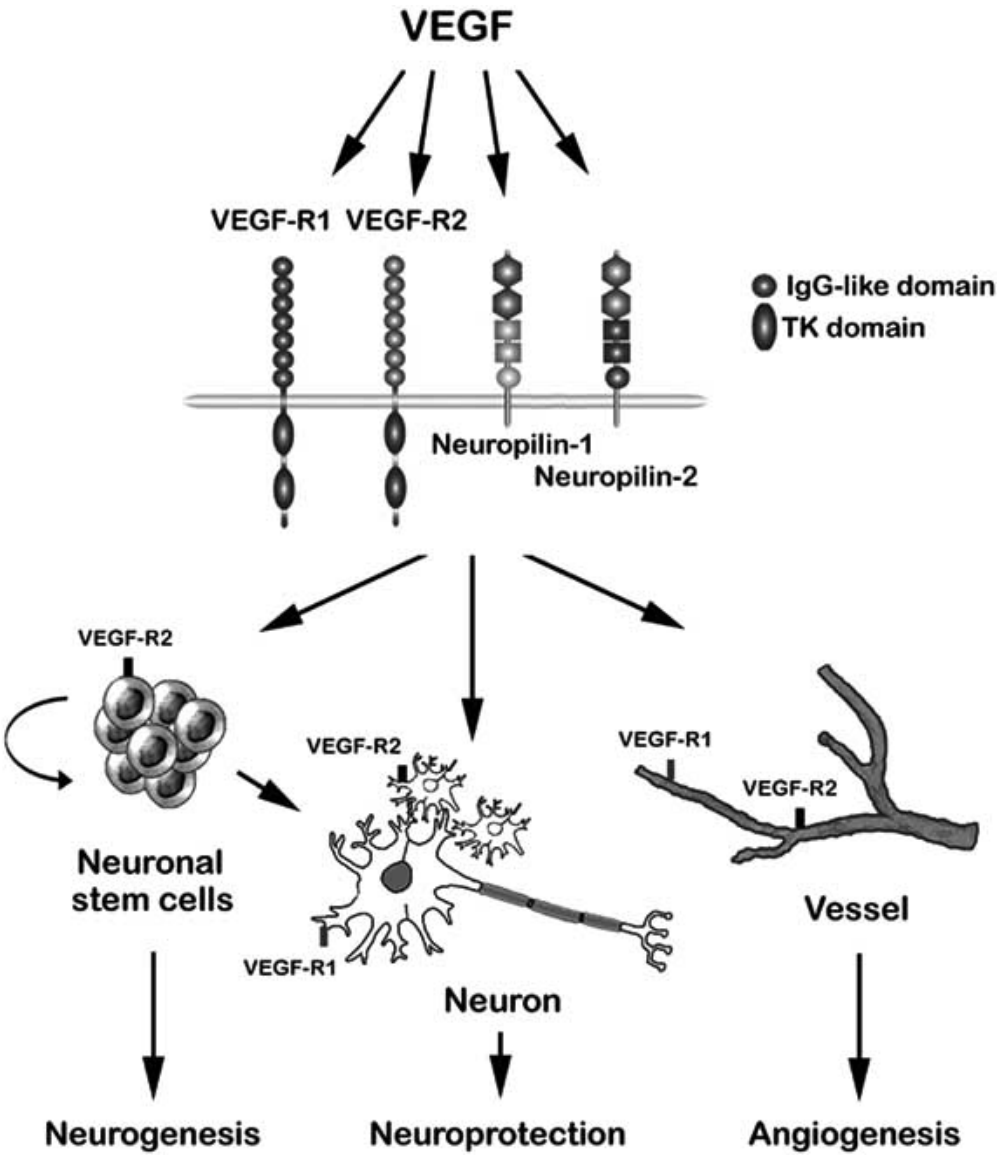

Fig. (1). A role of VEGF in angiogenesis, neuroprotection and neurogenesis.

augments neuronal precursor proliferation and enhances long-term survival of newborn neurons both in the SGZ and the SVZ [62].

While underlying cellular processes in neurogenesis activated by EGF, FGF-2, BDNF and erythropoietin have been documented, comparatively little is known about the mode of action of VEGF. VEGF binds to its tyrosine kinase receptors, VEGFR1/Flt1 and VEGFR2/Flk-1 (Fig. **1**), which are expressed not only in vascular tissues but also in neurons [63-65]. The available evidence suggests that the neurogenic effects of VEGF may be mediated by its binding to VEGFR2 [45, 60]. *In vitro*, the proliferative effect of VEGF on neuronal cultures is inhibited by inhibitors of classic proliferative signaling activities such as extracellular signal-regulated kinase kinase (MEK), phospholipase C (PLC), protein kinase C (PKC), and phosphatydilinositol 3-kinase (PI3K) [45, 66]. *In vivo*, protection against hypoxic neuronal injury by VEGF involves the inhibition of caspase-3 activity and enhancement of injury-induced neurogenesis in SVZ and SGZ [62, 67]. Interestingly, several recent reports have implicated other known neurogenic trophic factors such as BDNF [49, 68] and FGF-2 [42] in a VEGF–mediated pathway. It is thus possible that an important aspect of VEGF's mode of action is the release of other stimulatory growth factors, which may have dual role(s) in the angiogenic and neurogenic pathways activated by VEGF and mediate a feed-forward amplifying trophic effect.

Discovering how VEGF exerts its neurogenic and neuroprotective effects is likely to be of clinical relevance, as increased spontaneous adult neurogenesis may underlie the observed association between increased participation in intellectual, social and physical aspects of daily life and reduced risk of AD or slower cognitive decline [69] in the healthy elderly. Moreover, adult neurogenesis is stimulated by brain injury and may have a role in repair. VEGF has been implicated in the stimulation of neurogenesis in both scenarios. The ongoing investigation of VEGF's role in neurogenesis will allow us to define its preventive and remedial therapeutic potential.

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REFERENCES

[1] Bayer, S. A.; Yackel, J. W.; Puri, P. S. *Science*, **1982**, *216*, 890.
[2] Kaplan, M. S.; Hinds, J. W. *Science*, **1977**, *197*, 1092.
[3] Altman, J.; Das, G. D. *J. Comp. Neurol.*, **1965**, *124*, 319.
[4] Lois, C.; Garcia-Verdugo, J. M.; Alvarez-Buylla, A. *Science*, **1996**, *271*, 978.
[5] Temple, S.; Alvarez-Buylla, A. *Curr. Opin. Neurobiol.*, **1999**, *9*, 135.
[6] Kuhn, H. G.; Dickinson-Anson, H.; Gage, F. H. *J. Neurosci.*, **1996**, *16*, 2027.
[7] Kempermann, G.; Kuhn, H. G.; Gage, F. H. *Nature*, **1997**, *386*, 493.
[8] Maslov, A. Y.; Barone, T. A.; Plunkett, R. J.; Pruitt, S. C. *J. Neurosci.*, **2004**, *24*, 1726.
[9] Pencea, V.; Bingaman, K. D.; Freedman, L. J.; Luskin, M. B. *Exp. Neurol.*, **2001**, *172*, 1.
[10] Kukekov, V. G.; Laywell, E. D.; Suslov, O.; Davies, K.; Scheffler, B.; Thomas, L. B.;O'Brien, T. F.; Kusakabe, M.; Steindler, D. A. *Exp. Neurol.*, **1999**, *156*, 333.
[11] Eriksson, P. S.; Perfilieva, E.; Bjork-Eriksson, T. ;Alborn, A. M.; Nordborg, C.; Peterson, D. A.; Gage, F. H. *Nat. Med.*, **1998**, *4*, 1313.
[12] Alvarez-Buylla, A.; Lim, D. A. *Neuron*, **2004**, *41*, 683.
[13] Sommer, L.; Rao, M. *Prog. Neurobiol.*, **2002**, *66*, 1.
[14] Ohnuma, S.; Harris, W. A. *Neuron*, **2003**, *40*, 199.
[15] Doetsch, F.; Garcia-Verdugo, J. M.; Alvarez-Buylla, A. *J. Neurosci.*, **1997**, *17*, 5046.
[16] Bayer, S. A. *Exp. Brain. Res.*, **1983**, *50*, 329.
[17] Greenberg, D. A.; Jin, K. *Nat. Genet.*, **2004**, *36*, 792.
[18] Leung, D. W.; Cachianes, G.; Kuang, W. J.; Goeddel, D. V.; Ferrara, N. *Science*, **1989**, *246*, 1306.
[19] Carmeliet, P. *Nat. Med.*, **2003**, *9*, 653.
[20] Stein, I.; Neeman, M.; Shweiki, D.; Itin, A.; Keshet, E. *Mol. Cell. Biol.*, **1995**, *15*, 5363.
[21] Mukhopadhyay, D.; Tsiokas, L.; Zhou, X. M.; Foster, D.; Brugge, J. S.; Sukhatme, V. P. *Nature*, **1995**, *375*, 577.
[22] Brogi, E.; Schatteman, G.; Wu, T.; Kim, E. A.; Varticovski, L.; Keyt, B.; Isner, J. M. *J. Clin. Invest.*, **1996**, *97*, 469.
[23] Cao, Y.; Linden, P.; Shima, D.; Browne, F.; Folkman, J. *J. Clin. Invest.*, **1996**, *98*, 2507.
[24] Jin, K. L.; Mao, X. O.; Nagayama, T.; Goldsmith, P. C.; Greenberg, D. A. *Neuroscience*, **2000**, *99*, 577.
[25] Shweiki, D.; Itin, A.; Soffer, D.; Keshet, E. *Nature*, **1992**, *359*, 843.
[26] Weinstein, B. M. *Cell*, **2005**, *120*, 299.
[27] Mukouyama, Y. S.; Gerber, H. P.; Ferrara, N.; Gu, C.; Anderson, D. J. *Development*, **2005**, *132*, 941.
[28] Mukouyama, Y. S.; Shin, D.; Britsch, S.; Taniguchi, M.; Anderson, D. J. *Cell* **2002**, *109*, 693.
[29] Carmeliet, P.; Tessier-Lavigne, M. *Nature*, **2005**, *436*, 193.
[30] Carmeliet, P.; Ng, Y. S.; Nuyens, D.; Theilmeier, G.; Brusselmans, K.; Cornelissen, I.; Ehler, E.; Kakkar, V. V.; Stalmans, I.; Mattot, V.; Perriard, J. C.; Dewerchin, M.; Flameng, W.; Nagy, A.; Lupu, F.; Moons, L.; Collen, D.; D'Amore, P. A.; Shima, D. T. *Nat. Med.*, **1999**, *5*, 495.
[31] Serini, G.; Valdembri, D.; Zanivan, S.; Morterra, G.; Burkhardt, C.; Caccavari, F.; Zammataro, L.; Primo, L.; Tamagnone, L.; Logan, M.; Tessier-Lavigne, M.; Taniguchi, M.; Puschel, A. W.; Bussolino, F. *Nature*, **2003**, *424*, 391.
[32] Torres-Vazquez, J.; Gitler, A. D.; Fraser, S. D.; Berk, J. D.; Van, N. P.; Fishman, M. C.; Childs, S.; Epstein, J. A.; Weinstein, B. M. *Dev. Cell.*, **2004**, *7*, 117.
[33] Adams, R. H.; Wilkinson, G. A.; Weiss, C.; Diella, F.; Gale, N. W.; Deutsch, U.; Risau, W.; Klein, R. *Genes. Dev.*, **1999**, *13*, 295.
[34] Adams, R. H.; Diella, F.; Hennig, S.; Helmbacher, F.; Deutsch, U.; Klein, R. *Cell*, **2001**, *104*, 57.
[35] Lu, X.; Le Noble, F.; Yuan, L.; Jiang, Q.; De Lafarge, B.; Sugiyama, D.; Breant, C.; Claes, F.; De Smet, F.; Thomas, J. L.; Autiero, M.; Carmeliet, P.; Tessier-Lavigne, M.; Eichmann, A. *Nature*, **2004**, *432*, 179.
[36] Park, K. W.; Crouse, D.; Lee, M.; Karnik, S. K.; Sorensen, L. K.; Murphy, K. J.; Kuo, C. J.; Li, D. Y. *Proc. Natl. Acad. Sci. USA*, **2004**, *101*, 16210.
[37] Chen, H.; Chedotal, A.;He, Z.; Goodman, C. S.; Tessier-Lavigne, M. *Neuron*, **1997**, *19*, 547.
[38] He, Z.; Tessier-Lavigne, M. *Cell*, **1997**, *90*, 739.
[39] Soker, S.; Takashima, S.; Miao, H. Q.; Neufeld, G.; Klagsbrun, M. *Cell*, **1998**, *92*, 735.
[40] Yang, K.; Cepko, C. L. *J Neurosci.*, **1996**, *16*, 6089.
[41] Schwarz, Q.; Gu, C.; Fujisawa, H.; Sabelko, K.; Gertsenstein, M.; Nagy, A.; Taniguchi, M.; Kolodkin, A. L.; Ginty, D. D.; Shima, D. T.; Ruhrberg, C. *Genes. Dev.*, **2004**, *18*, 2822.
[42] Zhang, H.; Vutskits, L.; Pepper, M. S.; Kiss, J. Z. *J Cell Biol.*, **2003**, *163*, 1375.
[43] Sondell, M.; Lundborg, G.; Kanje, M. *J. Neurosci.*, **1999**, *19*, 5731.
[44] Silverman, W. F.; Krum, J. M.; Mani, N.; Rosenstein, J. M. *Neuroscience*, **1999**, *90*, 1529.
[45] Jin, K.; Mao, X. O.; Greenberg, D. A. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 10242.
[46] Jin, K.; Mao, X. O.; Greenberg, D. A. *J Mol Neurosci.*, **2000**, *14*, 197.
[47] Matsuzaki, H.; Tamatani, M.; Yamaguchi, A.; Namikawa, K.; Kiyama, H.; Vitek, M. P.; Mitsuda, N.; Tohyama, M. *FASEB. J.*, **2001**, *15*, 1218.
[48] Zhu, Y.; Jin, K.; Mao, X. O.; Greenberg, D. A. *FASEB. J.*, **2003**, *17*, 186.
[49] Louissaint, A., Jr.; Rao, S.; Leventhal, C.; Goldman, S. A. *Neuron*, **2002**, *34*, 945.
[50] Jin, K.; Zhu, Y.; Sun, Y.; Mao, X. O.; Xie, L.; Greenberg, D. A. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 11946.
[51] Palmer, T. D.; Willhoite, A. R.; Gage, F. H. *J. Comp. Neurol.*, **2000**, *425*, 479.
[52] Will, B.; Galani, R.; Kelche, C.; Rosenzweig, M. R. *Prog. Neurobiol.*, **2004**, *72*, 167.
[53] van Praag, H.; Kempermann, G.; Gage, F. H. *Nat. Neurosci.*, **1999**, *2*, 266.
[54] Trejo, J. L.; Carro, E.; Torres-Aleman, I. *J. Neurosci.*, **2001**, *21*, 1628.
[55] Carro, E.; Trejo, J. L.; Busiguina, S.; Torres-Aleman, I. *J Neurosci.*, **2001**, *21*, 5678.
[56] Campuzano, R.; Barrios, V.; Cuevas, B.; Asin-Cardiel, E.; Muela, A.; Castro, J. M.; Fernandez-Ayerdi, A.; Cuevas, P. *Eur. J. Med. Res.*, **2002**, *7*, 93.
[57] Schwarz, A. J.; Brasel, J. A.; Hintz, R. L.; Mohan, S.; Cooper, D. M. *J. Clin. Endocrinol. Metab.*, **1996**, *81*, 3492.
[58] Ickes, B. R.; Pham, T. M.; Sanders, L. A.; Albeck, D. S.; Mohammed, A. H.; Granholm, A. C. *Exp. Neurol.*, **2000**, *164*, 45.
[59] Fabel, K.; Tam, B.; Kaufer, D.; Baiker, A.; Simmons, N.; Kuo, C. J.; Palmer, T. D. *Eur. J. Neurosci.*, **2003**, *18*, 2803.
[60] Cao, L.; Jiao, X.; Zuzga, D. S.; Liu, Y.; Fong, D. M.; Young, D.; During, M. J. *Nat. Genet.*, **2004**, *36*, 827.
[61] Hayashi, T.; Abe, K.; Itoyama, Y. *J. Cereb. Blood. Flow. Metab.*, **1998**, *18*, 887.
[62] Sun, Y.; Jin, K.; Xie, L.; Childs, J.; Mao, X. O.; Logvinova, A.; Greenberg, D. A. *J. Clin. Invest.*, **2003**, *111*, 1843.
[63] Yang, X.; Cepko, C. L. *J. Neurosci.*, **1996**, *16*, 6089.
[64] Lennmyr, F.; Ata, K. A.; Funa, K.; Olsson, Y.; Terent, A. *J. Neuropathol. Exp. Neurol.*, **1998**, *57*, 874.
[65] Jin, K. L.; Mao, X. O.; Greenberg, D. A. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 10242.
[66] Ogunshola, O.O.; Antic, A.; Donoghune, M.J.; Fan, S.Y.; Kim, H.; Stewart, W.B.; Madri, J.A.; Ment, L.R. *J. Biol. Chem.*, **2002**, *277*, 11210-5
[67] Jin, K.; Minami, M.; Lan, J. Q.; Mao, X. O.; Batteur, S.; Simon, R. P.; Greenberg, D. A. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*, 4710.
[68] Chen, J.; Zacharek, A.; Zhang, C.; Jiang, H.; Li, Y.; Roberts, C.; Lu, M.; Kapke, A.; Chopp, M. *J. Neurosci.*, **2005**, *25*, 2366.
[69] Scarmeas, N.; Stern, Y. *Curr. Neurol. Neurosci. Rep.*, **2004**, *4*, 374.

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