Review Article

Angiogenic factors, bladder neuroplasticity and interstitial cystitis—new pathobiological insights

Ricardo Saban[1,2]

[1]University Anhembi Morumbi, S.Paulo, SP 03164-000, Brazil; [2]Department of Physiology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA

Correspondence to: Ricardo Saban, DVM, PhD. Director of Research and Postgraduation, University Anhembi Morumbi, Rua Dr. Almeida Lima, 1.134, sala 542, S. Paulo, S. Paulo CEP 03164-000, Brazil. Email: rssaban@Anhembi.br.

Abstract: Vascular endothelial growth factor (VEGF) is essential for normal embryonic development, and maintenance of adult vascular function. Originally described as a vascular permeability factor, VEGF alters tight cell junctions and contributes to maintenance of bladder permeability. VEGF and its receptors are not only expressed in bladder blood vessels but also in apical cells and intramural ganglia. VEGF receptors are fundamentally altered by inflammation and bladder diseases such as interstitial cystitis (IC). Experimental results indicate that VEGF exerts direct effects on bladder nerve density and function. Regardless of the etiology or initiating cause for IC, it is hypothesized that the urinary bladder responds to injury by increasing the production of VEGF that acts initially as a survival mechanism. However, VEGF also has the capacity to increase vascular permeability leading to glomerulations, edema, and inflammation. Moreover, due to elevated numbers of VEGF receptors in the urothelium, the increased levels of VEGF further increase bladder permeability and establish a vicioCus cycle of disease pathophysiology.

Keywords: Neuropilins (NRPs); vascular endothelial growth factor (VEGF); neuroplasticity; hormones; pregnancy; soluble VEGF receptors

Submitted Apr 15, 2015. Accepted for publication Aug 03, 2015.
doi: 10.3978/j.issn.2223-4683.2015.08.05
View this article at: http://dx.doi.org/10.3978/j.issn.2223-4683.2015.08.05

Interstitial cystitis/painful bladder syndrome (IC/PBS)

IC/PBS is a chronic and painful syndrome of unknown cause with no reliable biologic marker or effective therapy. This debilitating disorder has, in addition to pain, symptoms of pressure or discomfort, and an urgent need to urinate day and night. Many patients experience a worsening of their symptoms due to emotional or physical stress. Pain, frequency and urgency, and the lack of sleep experienced by IC patients may themselves be significant causes of stress and therefore, may contribute to perpetuation of symptoms.

The NIH-NIDDK Interstitial Cystitis Database Study in the US found that the following pathological features are associated with IC: (I) mast cell count in lamina propria on tryptase stain; (II) loss of urothelium; (III) granulation tissue in lamina propria; and (IV) vascular density in lamina propria on factor VIII (F8) stain. Finally, the percentage of mucosa denuded of urothelium and the percentage of submucosal hemorrhage were highly associated with pain in a multivariate predictive model (1).

Vascular system and IC

The vascular system appears to be involved in the development of IC (1). Indeed, one of the major vascular alterations in IC patients, during bladder examination by endoscopy, is the presence of bladder wall glomerulations (pinpoint bleeding) indicating blood vessel fragility (2-4). This vascular injury seems to initiate at the epithelial/urothelial surface and propagates towards the interstitium, causing secondary involvement of the microvasculature (5). It has to be taken in consideration that most of

the glomerulation is observed subsequent bladder overdistention and studies pointed out that the cystoscopic appearance of the bladder wall after hydrodistention may not be constant over time, and the absence of initial findings of glomerulations or terminal hematuria does not preclude further development of these hallmarks of the disease on subsequent evaluation (6). It must be noted that most studies that failed to find a relationship between symptom reports and cystoscopic findings were performed with patients undergoing treatment for IC. In this context, a very interesting study compared symptoms and cystoscopic findings in patients with untreated IC with those reported in the literature obtained during treatment (7). In untreated IC patients, pain had consistent positive correlations with the cystoscopic findings and the increase in pain with bladder filling was associated with inflammation, ulceration, and smaller bladder capacity. In addition, pain intensity was related to a smaller bladder capacity and the presence of glomerulations (7).

Investigators have raised the possibility that the glomerulations seen in IC patients could be related to endogenous factors. A putative candidate is the vascular endothelial growth factor (VEGF), originally described as a permeability factor by Dvorak and colleagues, a factor that, contributes to vascular pathobiology (8). Generally, VEGF has been associated with angiogenesis and with lymphangiogenesis (9). However, recent evidence indicates that VEGF receptors are expressed in cells other than the vascular endothelium, and that VEGF also elicits other responses besides increased endothelial permeability and cell migration (10). VEGF receptors are present in vascular smooth muscle cells (11), osteoblasts (12), cardiac myocytes (13), myofibroblasts (14), neurons (15), and various tumor cells (16). VEGF is described to protect neurons from ischemic injury (17,18) and is a survival factor for renal tubular epithelial cells (19), endothelial cells (20), stem cells (21), and neuropilin (NRP)-positive cancer cells (22).

VEGF signaling is a central component of molecular signaling pathways associated with bladder inflammation (23-25), and a key downstream mechanism of inflammation induced by activation of protease-activated receptors (26). This new appreciation of VEGF signaling in bladder inflammation is supported by the emerging evidence that levels of various VEGFs are increased at the site of inflammation (27-31), and that infiltrating lymphocytes and other inflammatory cells represent additional sources of VEGF (30,32).

VEGF has been intensively studied with respect to its actions on vascular endothelial cells, and in the bladder, increased staining of VEGF was reported in patients with glomerulations on hydrodistention, but not in patients who failed to show petechial bleeding or in controls (33). Indeed, Tamaki *et al.* reported that glomerulations were highly associated with the elevated levels of VEGF (33). Based on Tamaki's work, the expression of VEGF receptors in bladder biopsies of IC patients were assessed and fundamental alterations in the receptors were reported (34). Striking differences between IC and control bladders were found in the blood vessels, the urothelial cells lining the bladder surface and VEGF receptors in intramural ganglia (34). Other investigators also reported that VEGF proteins were increased in biopsies of IC patients compared with the controls and that hypoxia-inducible factor-1 was also elevated (35). The overexpression of VEGF was particularly evident in umbrella cells examined by confocal microscopy (35). High levels of VEGF have been shown to induce immature angiogenesis, where microvessels have insufficient coverage of pericytes, resulting in hemorrhagic vessels. Moreover, in IC biopsies, increased levels VEGF were associated with immature vascularization (36). Interestingly, a surprising revelation was that VEGF expression was associated with the degree of pain described by patients (36). Treatments and procedures that reduce VEGF levels in the bladder seem to be effective in reducing the symptoms of IC in humans. Intravesical botulinum toxin A injection combined with hydrodistention significantly decreased VEGF levels and was associated with a decrease in apoptotic cell count and mast cell activity (37). Interestingly classical treatments for IC such as pentosan polysulfate also reduce VEGF levels in cell lines (38).

Another aspect of VEGF research in IC is based on the finding that acute stress worsens IC symptoms. What is now clear is that stress, and changes in corticotrophin-releasing hormone (CRH) receptor (CRH-R) are correlated with VEGF levels. Acute stress increased bladder vascular permeability and VEGF release that is dependent on CRH-R (39). Another interesting aspect of VEGF research is that, in mast cells, CRH receptors are selectively associated with VEGF secretion (35). These findings together suggest that stress, CRH, mast cells and VEGF might participate in the pathogenesis of PBS/IC (39).

Neuropilins (NRPs) and the urinary system

NRPs were initially identified as co-receptors for plexin and mediating the responses of semaphorin on axon guidance

and organ innervation (40). However, NRPs functions are also co-receptors for VEGF and enhance binding to VEGF receptors (41). Two related NRPs, NRP1 and NRP2, are at the heart of the cross-talk between the nervous and vascular systems (42). Through the action of semaphorin, NRPs control the density of innervation whereas through VEGF, NRPs guide vascular growth. Recent evidence indicates that NRPs are expressed outside of the vascular system (43) and play a fundamental role in the activation of inflammatory cells, antigen presenting cells (44), effector cells (45,46), and cancer cells (47).

Visualization of VEGF and NRP receptors with infrared fluorescence (NIRF)

In order to determine the distribution of VEGF and NRPs in the urinary bladder, we utilized NIRF because it has high photon penetration and low auto-fluorescence in the 700-900 nm wavelength range (48-51). Light in the near infrared spectrum efficiently traverses biological tissue, as the absorption of water and hemoglobin is very low in that spectrum. In addition, NIRF was greatly enhanced with the advent of cyanine (Cy) dyes—optical contrast agents with nearly ideal properties including high extinction coefficients, and absorption and emission ranges throughout the visible near infrared spectrum (52-54). To determine the nature of cells responding to VEGF in normal and inflamed bladders, a fluorescent tracer, scVEGF/Cy, an engineered single-chain VEGF labeled with Cy5.5 dye was used to identify cells with accessible and functionally active VEGF receptors (55). Importantly, unlike immunohistochemical analysis that shows all cells expressing VEGF receptors, receptor-mediated tagging with scVEGF/Cy tracer identifies only cells with functionally active VEGF receptors (54,56,57). Accumulation of scVEGF/Cy in the urothelium was co-localized with cells expressing NRPs and VEGF receptors (55). NRPs were found to be highly expressed in the mouse bladder urothelium and intramural ganglia, and up-regulated during experimental bladder inflammation (55) and in cyclophosphamide-induced cystitis (58).

In addition to the mouse bladder, expression of NRP was also found highly expressed the human urothelium carcinoma cell line (J82) (34), and human bladder biopsies (55). Others have shown an intense expression of NRP in the mouse bladder detrusor muscle at E15.5 (40) by *in situ* hybridization and NRP2 was among the top ranked molecular target differentially expressed in human bladder cancer (55).

NRPs and uroplakins

As NRPs are highly expressed in the mouse and human bladder urothelium, it is interesting to note these molecules are co-localized with integrins in parts of the cell, primarily in tetraspanin-enriched microdomains (59). Induction of NRP expression enables the formation of an NRP-integrin complex that regulates the function in response to VEGF stimulation (59). At a functional level, the association between integrins and NRPs was shown to be important for the endocytic trafficking of integrin, resulting in increased cell adhesion to fibronectin (59). An alternate hypothesis for the co-localization of NRPs and integrin, albeit speculative, is that integrins can interact with either tetraspanins or NRPs and those interactions have distinct functional consequences (59). The association of NRPs with tetraspanin molecules raise the question of whether NRPs found in the urothelium are associated with the expression of other members of the tetraspanins family molecules like uroplakins and, therefore, whether their expression are regulated by the same mechanisms.

Nerve and blood vessel development are associated

It is not coincidental that VEGF and NRPs are in the center of alterations of both blood vessels and nerves. Evidence has shown that: (I) nerves and blood vessels are anatomically associated; (II) follow a common molecular pathway during development; and (III) their maturation in adulthood may be controlled by the same key molecules responsible for their development (60,61). The finding that mutant mice (neurogenin 1/neurogenin 2 double knockout embryos) lacking sensory nerves also have disorganized blood vessel branching (62), suggests that local signals supplied by nerve fibers, may provide a cue that determines blood vessel patterning. The new hypothesis is that many proteins that were originally discovered to be required for axon guidance are implicated in the development of the vascular (61) and lymphatic systems (63). Perhaps the most striking observation linking the nervous and vascular systems is the finding that angiogenic factors such as VEGF, when deregulated, contribute to various neurological disorders, including neurodegeneration (64-66). VEGF is a prototypic example of a cross-talk between nerves and vessels (67). Although originally described as a key angiogenic and permeability factor, it is now well established that VEGF also plays a crucial role in the development of the nervous

system (67). VEGF is now described to participate in the time course of vessel maturation and alterations in his pathway determines vulnerability to neuronal injuries (68).

What would be the role of VEGF on nerves?

It is now known that VEGF is necessary to maintain a healthy adult blood circulation, as reduced VEGF activity is followed by numerous blood vessels abnormalities (69) that can be reversed by local VEGF administration (70). It seems that VEGF has the same impact on nerves. Evidence indicates that VEGF exerts direct neuroprotective effects through its receptors, a finding that suggests a clinically relevant role for VEGF in preventing distal neuropathies (71). Moreover, VEGF enhances intraneural angiogenesis and improves nerve regeneration (72-75).

Does VEGF also alter bladder nerve density and function?

We were intrigued by evidence that chronic inflammation increases the density of bladder sensory nerves that express: (I) the capsaicin transient receptor potential vanilloid subfamily 1 (TRPV1) (76); (II) protein gene product (PGP9.5) (77); (III) substance P; and (IV) calcitonin gene-related peptide (CGRP) (25). We also determined that a VEGF neutralizing antibody (B20) prevented inflammation-induced increase in sensory nerve density (25). Furthermore, instillation of VEGF into the mouse bladder recapitulated the effect of inflammation on sensory nerve plasticity and represents direct evidence of VEGF action on the peripheral nervous system (25). We sought to determine whether VEGF, in addition to increasing in sensory nerve density, also alters the density of cholinergic nerves, and, consequently, bladder function and visceral sensitivity. Our results indicate that, in addition to an overwhelming increase in TRPV1, VEGF instillation resulted in an increase in choline acetyltransferase (ChAT) expression in several layers of the urinary bladder wall (25). We also tested whether alterations in nerve density was reflected by a concomitant change in bladder function. Indeed, intravesical VEGF caused a profound change in the function of the urinary bladder: acute VEGF (1 week post-VEGF treatment) reduced micturition pressure and longer treatment (2 weeks post-VEGF instillation) caused a substantial reduction in inter-micturition interval (25) which is characteristic of bladder disorders. In addition, intravesical VEGF resulted in an up-regulation of voltage gated Na^+ channels in bladder dorsal root ganglia neurons and enhanced abdominal sensitivity to mechanical stimulation (25).

Endogenous VEGF antagonists

An association between IC and sex hormones has been proposed based on the disproportional incidence of this disease in women and because early findings indicate that the female sexual hormones may interfere with the progression of IC (78,79). An interesting idea regarding IC development is based on the existence of an endogenous soluble VEGF receptor (sFLT1) produced by the placenta which neutralizes VEGF (80). Although VEGF is essential for normal embryonic development, it has been shown that mild elevation of local VEGF levels during early pregnancy can cause severe placental vascular damage, blood vessel leaking, and embryonic lethality (80). Moreover, modest local increases in VEGF could also be a primary trigger for elevation of placental sFLT1 expression, leading to the hallmark symptoms of preeclampsia (81). This breakthrough work lead to the notion that placental sFLT1 plays an essential role in placental functions. Overexpression of sFLT1 in preeclampsia, although damaging to the mother, serves a critical protective function for the placenta and fetus through its sequestration of maternal VEGF (81). In conclusion, mild deviations from normal VEGF and sFLT1 levels during pregnancy could have serious consequences to the mother's body (81). A growing body of observations, including the side effects of anti-VEGF therapies as well as the role sFLT1 in preeclampsia, points to an important role for VEGF in maintenance of stable adult blood vessels. It is interesting to note that kidney circulation is fundamentally altered in pre-eclampsia (80). A question arises in form of speculation: during pre-eclampsia, in addition to alterations observed in the kidneys would high levels of sFLT1 damage the urinary bladder vasculature? Such alterations in sFLT1 levels would represent a link between of hormonal fluctuation and pregnancy and bladder pathology.

Our hypothesis is that regardless of the cause for IC, the urinary bladder responds to injury by increasing the production of VEGF that acts initially as a survival factor. However, VEGF also can increase vascular permeability leading to glomerulations, edema, and inflammation. Moreover, due to elevated numbers of VEGF receptors in the urothelium, the increased levels of VEGF further increase bladder permeability and establish a vicious cycle that perpetuates the disease. Four lines of evidence suggest

that VEGF/NRP signaling could be aberrant in IC. First, the urothelium is "leaky" in IC, and VEGF signaling alters not only vascular permeability (82) but also disrupts tight junction proteins (83-88). Second is the evidence for abnormal capillary growth in IC (89-91). Third is the hypothesis for a connection between neural and epithelial function (92), an action that in other systems could be modulated by NRPs. Fourth, NRP and VEGF receptor expression is altered in the bladder urothelium of IC patients (34).

Another interesting hypothesis suggested from the above findings is that increased bladder VEGF is also responsible for altered bladder nerve plasticity, pain and function. Therefore, blocking VEGF signaling pathway should reduce the basic mechanisms involved in the genesis and symptoms of IC. In order to answer this relevant question, investigators have an arsenal of FDA approved drugs known to alter the VEGF pathway that are clinically used in humans with cancer. These agents may be worthy of study for the treatment of IC!

Acknowledgements

Funding: This entire research was supported by the Department of Defense Medical Research Program (PRMRP) under award number PR080981. Views and opinions of, and endorsements by the author(s) do not reflect those of the US Army or the Department of Defense. Preliminary results were support in part by NIH-NIDDK award number 2-R56-DK66101.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Tomaszewski JE, Landis JR, Russack V, et al. Biopsy features are associated with primary symptoms in interstitial cystitis: results from the interstitial cystitis database study. Urology 2001;57:67-81.
2. Andersson KE, Hedlund P. Pharmacologic perspective on the physiology of the lower urinary tract. Urology 2002;60:13-20; discussion 20-1.
3. Mattila J, Pitkanen R, Vaalasti T, et al. Fine-structural evidence for vascular injury in patients with interstitial cystitis. Virchows Arch A Pathol Anat Histopathol 1983;398:347-55.
4. Cheng C, Rosamilia A, Healey M. Diagnosis of interstitial cystitis/bladder pain syndrome in women with chronic pelvic pain: a prospective observational study. Int Urogynecol J 2012;23:1361-6.
5. Járomi P, Szabó A, Garab D, et al. Experimental studies on microcirculatory inflammatory reactions of the urinary bladder. Magy Seb 2012;65:184-90.
6. Shear S, Mayer R. Development of glomerulations in younger women with interstitial cystitis. Urology 2006;68:253-6.
7. Lamale LM, Lutgendorf SK, Hoffman AN, et al. Symptoms and cystoscopic findings in patients with untreated interstitial cystitis. Urology 2006;67:242-5.
8. Senger DR, Galli SJ, Dvorak AM, et al. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 1983;219:983-5.
9. Tamarkin FJ, Kang WS, Cohen JJ, et al. A role for Akt in the rapid regulation of inflammatory and apoptotic pathways in mouse bladder. Naunyn Schmiedebergs Arch Pharmacol 2006;373:349-59.
10. Weis SM, Cheresh DA. Pathophysiological consequences of VEGF-induced vascular permeability. Nature 2005;437:497-504.
11. Ishida A, Murray J, Saito Y, et al. Expression of vascular endothelial growth factor receptors in smooth muscle cells. J Cell Physiol 2001;188:359-68.
12. Deckers MM, Karperien M, van der Bent C, et al. Expression of vascular endothelial growth factors and their receptors during osteoblast differentiation. Endocrinology 2000;141:1667-74.
13. Takahashi N, Seko Y, Noiri E, et al. Vascular endothelial growth factor induces activation and subcellular translocation of focal adhesion kinase (p125FAK) in cultured rat cardiac myocytes. Circ Res 1999;84:1194-202.
14. Chintalgattu V, Nair DM, Katwa LC. Cardiac myofibroblasts: a novel source of vascular endothelial growth factor (VEGF) and its receptors Flt-1 and KDR. J Mol Cell Cardiol 2003;35:277-86.
15. Carmeliet P, Storkebaum E. Vascular and neuronal effects of VEGF in the nervous system: implications for neurological disorders. Semin Cell Dev Biol 2002;13:39-53.
16. Xie K, Wei D, Shi Q, et al. Constitutive and inducible expression and regulation of vascular endothelial growth factor. Cytokine Growth Factor Rev 2004;15:297-324.
17. Feng Y, Rhodes PG, Bhatt AJ. Neuroprotective effects of vascular endothelial growth factor following hypoxic ischemic brain injury in neonatal rats. Pediatr Res 2008;64:370-4.

18. Nishijima K, Ng YS, Zhong L, et al. Vascular endothelial growth factor-A is a survival factor for retinal neurons and a critical neuroprotectant during the adaptive response to ischemic injury. Am J Pathol 2007;171:53-67.
19. Kanellis J, Fraser S, Katerelos M, et al. Vascular endothelial growth factor is a survival factor for renal tubular epithelial cells. Am J Physiol Renal Physiol 2000;278:F905-15.
20. Ilan N, Mahooti S, Madri JA. Distinct signal transduction pathways are utilized during the tube formation and survival phases of in vitro angiogenesis. J Cell Sci 1998;111:3621-31.
21. Brusselmans K, Bono F, Collen D, et al. A novel role for vascular endothelial growth factor as an autocrine survival factor for embryonic stem cells during hypoxia. J Biol Chem 2005;280:3493-9.
22. Bachelder RE, Crago A, Chung J, et al. Vascular endothelial growth factor is an autocrine survival factor for neuropilin-expressing breast carcinoma cells. Cancer Res 2001;61:5736-40.
23. Pavlovich CP, Kraling BM, Stewart RJ, et al. BCG-induced urinary cytokines inhibit microvascular endothelial cell proliferation. J Urol 2000;163:2014-21.
24. Saban MR, Hellmich H, Nguyen NB, et al. Time course of LPS-induced gene expression in a mouse model of genitourinary inflammation. Physiol Genomics 2001;5:147-60.
25. Saban MR, Davis CA, Avelino A, et al. VEGF signaling mediates bladder neuroplasticity and inflammation in response to BCG. BMC Physiol 2011;11:16.
26. Saban MR, Nguyen NB, Hammond TG, et al. Gene expression profiling of mouse bladder inflammatory responses to LPS, substance P, and antigen-stimulation. Am J Pathol 2002;160:2095-110.
27. Visvanathan S, Wagner C, Marini JC, et al. Inflammatory biomarkers, disease activity and spinal disease measures in patients with ankylosing spondylitis after treatment with infliximab. Ann Rheum Dis 2008;67:511-7.
28. Rovina N, Papapetropoulos A, Kollintza A, et al. Vascular endothelial growth factor: an angiogenic factor reflecting airway inflammation in healthy smokers and in patients with bronchitis type of chronic obstructive pulmonary disease? Respir Res 2007;8:53.
29. Futami R, Miyashita M, Nomura T, et al. Increased serum vascular endothelial growth factor following major surgical injury. J Nippon Med Sch 2007;74:223-9.
30. Halin C, Tobler NE, Vigl B, et al. VEGF-A produced by chronically inflamed tissue induces lymphangiogenesis in draining lymph nodes. Blood 2007;110:3158-67.
31. Clavel G, Bessis N, Lemeiter D, et al. Angiogenesis markers (VEGF, soluble receptor of VEGF and angiopoietin-1) in very early arthritis and their association with inflammation and joint destruction. Clin Immunol 2007;124:158-64.
32. Freeman MR, Schneck FX, Gagnon ML, et al. Peripheral blood T lymphocytes and lymphocytes infiltrating human cancers express vascular endothelial growth factor: a potential role for T cells in angiogenesis. Cancer Res 1995;55:4140-5.
33. Tamaki M, Saito R, Ogawa O, et al. Possible mechanisms inducing glomerulations in interstitial cystitis: relationship between endoscopic findings and expression of angiogenic growth factors. J Urol 2004;172:945-8.
34. Saban R, Saban MR, Maier J, et al. Urothelial expression of Neuropilins and VEGF receptors in control and interstitial cystitis patients. Am J Physiol Renal Physiol 2008. Available online: http://ajprenal.physiology.org/content/ajprenal/early/2008/09/24/ajprenal.90344.2008.full.pdf
35. Boucher W, Kempuraj D, Michaelian M, et al. Corticotropin-releasing hormone-receptor 2 is required for acute stress-induced bladder vascular permeability and release of vascular endothelial growth factor. BJU Int 2010;106:1394-9.
36. Kiuchi H, Tsujimura A, Takao T, et al. Increased vascular endothelial growth factor expression in patients with bladder pain syndrome/interstitial cystitis: its association with pain severity and glomerulations. BJU Int 2009;104:826-31; discussion 31.
37. Peng CH, Jhang JF, Shie JH, et al. Down regulation of vascular endothelial growth factor is associated with decreased inflammation after intravesical OnabotulinumtoxinA injections combined with hydrodistention for patients with interstitial cystitis--clinical results and immunohistochemistry analysis. Urology 2013;82:1452.e1-6.
38. Zaslau S, Sparks S, Riggs D, et al. Pentosan polysulfate (Elmiron): in vitro effects on prostate cancer cells regarding cell growth and vascular endothelial growth factor production. Am J Surg 2006;192:640-3.
39. Cao J, Boucher W, Kempuraj D, et al. Acute stress and intravesical corticotropin-releasing hormone induces mast cell dependent vascular endothelial growth factor release from mouse bladder explants. J Urol 2006;176:1208-13.
40. Chen H, Chedotal A, He Z, et al. Neuropilin-2, a novel member of the neuropilin family, is a high affinity receptor for the semaphorins Sema E and Sema IV but not Sema

III. Neuron 1997;19:547-59.
41. Soker S, Takashima S, Miao HQ, et al. Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell 1998;92:735-45.
42. Eichmann A, Makinen T, Alitalo K. Neural guidance molecules regulate vascular remodeling and vessel navigation. Genes Dev 2005;19:1013-21.
43. Pellet-Many C, Frankel P, Jia H, et al. Neuropilins: structure, function and role in disease. Biochem J 2008;411:211-26.
44. Bourbié-Vaudaine S, Blanchard N, Hivroz C, et al. Dendritic cells can turn CD4+ T lymphocytes into vascular endothelial growth factor-carrying cells by intercellular neuropilin-1 transfer. J Immunol 2006;177:1460-9.
45. Battaglia A, Buzzonetti A, Monego G, et al. Neuropilin-1 expression identifies a subset of regulatory T cells in human lymph nodes that is modulated by preoperative chemoradiation therapy in cervical cancer. Immunology 2008;123:129-38.
46. Bruder D, Probst-Kepper M, Westendorf AM, et al. Neuropilin-1: a surface marker of regulatory T cells. Eur J Immunol 2004;34:623-30.
47. Caunt M, Mak J, Liang WC, et al. Blocking neuropilin-2 function inhibits tumor cell metastasis. Cancer Cell 2008;13:331-42.
48. Sevick-Muraca EM, Houston JP, Gurfinkel M. Fluorescence-enhanced, near infrared diagnostic imaging with contrast agents. Curr Opin Chem Biol 2002;6:642-50.
49. Ntziachristos V, Bremer C, Weissleder R. Fluorescence imaging with near-infrared light: new technological advances that enable in vivo molecular imaging. Eur Radiol 2003;13:195-208.
50. Frangioni JV. In vivo near-infrared fluorescence imaging. Curr Opin Chem Biol 2003;7:626-34.
51. Kim S, Lim YT, Soltesz EG, et al. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. Nat Biotechnol 2004;22:93-7.
52. Ntziachristos V, Schellenberger EA, Ripoll J, et al. Visualization of antitumor treatment by means of fluorescence molecular tomography with an annexin V-Cy5.5 conjugate. Proc Natl Acad Sci U S A 2004;101:12294-9.
53. Ntziachristos V, Bremer C, Graves EE, et al. In vivo tomographic imaging of near-infrared fluorescent probes. Mol Imaging 2002;1:82-8.
54. Backer MV, Gaynutdinov TI, Patel V, et al. Vascular endothelial growth factor selectively targets boronated dendrimers to tumor vasculature. Mol Cancer Ther 2005;4:1423-9.
55. Saban MR, Backer JM, Backer MV, et al. VEGF receptors and neuropilins are expressed in the urothelial and neuronal cells in normal mouse urinary bladder and are up-regulated in inflammation. Am J Physiol Renal Physiol 2008;295:F60-72.
56. Backer MV, Backer JM. Functionally active VEGF fusion proteins. Protein Expr Purif 2001;23:1-7.
57. Backer MV, Levashova Z, Patel V, et al. Molecular imaging of VEGF receptors in angiogenic vasculature with single-chain VEGF based probes. Nat Med 2007;13:504-9.
58. Cheppudira BP, Girard BM, Malley SE, et al. Upregulation of vascular endothelial growth factor isoform VEGF-164 and receptors (VEGFR-2, Npn-1, and Npn-2) in rats with cyclophosphamide-induced cystitis. Am J Physiol Renal Physiol 2008;295:F826-36.
59. Goel HL, Mercurio AM. Enhancing integrin function by VEGF/neuropilin signaling: implications for tumor biology. Cell Adh Migr 2012;6:554-60.
60. Martin P, Lewis J. Origins of the neurovascular bundle: interactions between developing nerves and blood vessels in embryonic chick skin. Int J Dev Biol 1989;33:379-87.
61. Carmeliet P, Tessier-Lavigne M. Common mechanisms of nerve and blood vessel wiring. Nature 2005;436:193-200.
62. Mukouyama YS, Shin D, Britsch S, et al. Sensory nerves determine the pattern of arterial differentiation and blood vessel branching in the skin. Cell 2002;109:693-705.
63. Yuan L, Moyon D, Pardanaud L, et al. Abnormal lymphatic vessel development in neuropilin 2 mutant mice. Development 2002;129:4797-806.
64. Ruan L, Wang B, ZhuGe Q, et al. Coupling of neurogenesis and angiogenesis after ischemic stroke. Brain Res 2015;1623:166-73.
65. Lin AJ, Liu G, Castello NA, et al. Optical imaging in an Alzheimer's mouse model reveals amyloid--dependent vascular impairment. Neurophotonics 2014;1:011005.
66. Pronto-Laborinho AC, Pinto S, de Carvalho M. Roles of vascular endothelial growth factor in amyotrophic lateral sclerosis. Biomed Res Int 2014;2014:947513.
67. Ruiz de Almodovar C, Lambrechts D, Mazzone M, et al. Role and therapeutic potential of VEGF in the nervous system. Physiol Rev 2009;89:607-48.
68. Licht T, Dor-Wollman T, Ben-Zvi A, et al. Vessel maturation schedule determines vulnerability to neuronal injuries of prematurity. J Clin Invest 2015;125:1319-28.
69. Storkebaum E, Ruiz de Almodovar C, Meens M, et al. Impaired autonomic regulation of resistance arteries

in mice with low vascular endothelial growth factor or upon vascular endothelial growth factor trap delivery. Circulation 2010;122:273-81.
70. Chade AR, Kelsen S. Reversal of renal dysfunction by targeted administration of VEGF into the stenotic kidney: a novel potential therapeutic approach. Am J Physiol Renal Physiol 2012;302:F1342-50.
71. Dhondt J, Peeraer E, Verheyen A, et al. Neuronal FLT1 receptor and its selective ligand VEGF-B protect against retrograde degeneration of sensory neurons. FASEB J 2011;25:1461-73.
72. Hobson MI, Green CJ, Terenghi G. VEGF enhances intraneural angiogenesis and improves nerve regeneration after axotomy. J Anat 2000;197:591-605.
73. Pereira Lopes FR, Lisboa BC, Frattini F, et al. Enhancement of sciatic nerve regeneration after vascular endothelial growth factor (VEGF) gene therapy. Neuropathol Appl Neurobiol 2011;37:600-12.
74. Pola R, Aprahamian TR, Bosch-Marce M, et al. Age-dependent VEGF expression and intraneural neovascularization during regeneration of peripheral nerves. Neurobiol Aging 2004;25:1361-8.
75. Tang J, Hua Y, Su J, et al. Expression of VEGF and neural repair after alprostadil treatment in a rat model of sciatic nerve crush injury. Neurol India 2009;57:387-94.
76. Charrua A, Reguenga C, Cordeiro JM, et al. Functional transient receptor potential vanilloid 1 is expressed in human urothelial cells. J Urol 2009;182:2944-50.
77. Doran JF, Jackson P, Kynoch PA, et al. Isolation of PGP 9.5, a new human neurone-specific protein detected by high-resolution two-dimensional electrophoresis. J Neurochem 1983;40:1542-7.
78. Warren JW, Clauw DJ, Wesselmann U, et al. Sexuality and reproductive risk factors for interstitial cystitis/painful bladder syndrome in women. Urology 2011;77:570-5.
79. Powell-Boone T, Ness TJ, Cannon R, et al. Menstrual cycle affects bladder pain sensation in subjects with interstitial cystitis. J Urol 2005;174:1832-6.
80. Adamson SL. sFLT1 in preeclampsia: trophoblast defense against a decidual VEGFA barrage? J Clin Invest 2014;124:4690-2.
81. Fan X, Rai A, Kambham N, et al. Endometrial VEGF induces placental sFLT1 and leads to pregnancy complications. J Clin Invest 2014;124:4941-52.
82. Rosamilia A, Dwyer PL. Pathophysiology of interstitial cystitis. Curr Opin Obstet Gynecol 2000;12:405-10.
83. Schmitt M, Horbach A, Kubitz R, et al. Disruption of hepatocellular tight junctions by vascular endothelial growth factor (VEGF): a novel mechanism for tumor invasion. J Hepatol 2004;41:274-83.
84. Rodewald M, Herr D, Fraser HM, et al. Regulation of tight junction proteins occludin and claudin 5 in the primate ovary during the ovulatory cycle and after inhibition of vascular endothelial growth factor. Mol Hum Reprod 2007;13:781-9.
85. Fraser MO, Chuang YC, Lavelle JP, et al. A reliable, nondestructive animal model for interstitial cystitis: intravesical low-dose protamine sulfate combined with physiological concentrations of potassium chloride. Urology 2001;57:112.
86. Nico B, Mangieri D, Crivellato E, et al. HIF activation and VEGF overexpression are coupled with ZO-1 up-phosphorylation in the brain of dystrophic mdx mouse. Brain Pathol 2007;17:399-406.
87. Peters S, Cree IA, Alexander R, et al. Angiopoietin modulation of vascular endothelial growth factor: Effects on retinal endothelial cell permeability. Cytokine 2007;40:144-50.
88. Ghassemifar R, Lai CM, Rakoczy PE. VEGF differentially regulates transcription and translation of ZO-1alpha+ and ZO-1alpha- and mediates trans-epithelial resistance in cultured endothelial and epithelial cells. Cell Tissue Res 2006;323:117-25.
89. Rosamilia A, Igawa Y, Higashi S. Pathology of interstitial cystitis. Int J Urol 2003;10:S11-5.
90. Rosamilia A, Cann L, Scurry J, et al. Bladder microvasculature and the effects of hydrodistention in interstitial cystitis. Urology 2001;57:132.
91. Rosamilia A, Cann L, Dwyer P, et al. Bladder microvasculature in women with interstitial cystitis. J Urol 1999;161:1865-70.
92. Apodaca G, Kiss S, Ruiz W, et al. Disruption of bladder epithelium barrier function after spinal cord injury. Am J Physiol Renal Physiol 2003;284:F966-76.

Cite this article as: Saban R. Angiogenic factors, bladder neuroplasticity and interstitial cystitis—new pathobiological insights. Transl Androl Urol 2015;4(5):555-562. doi: 10.3978/j.issn.2223-4683.2015.08.05

