Review Article

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

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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

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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

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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

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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

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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.

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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

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