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REVIEW

Three important components in the regeneration of the cavernous nerve: brain-derived neurotrophic factor, vascular endothelial growth factor and the JAK/STAT signaling pathway

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Retroperitoneal operations, such as radical prostatectomy, often damage the cavernous nerve, resulting in a high incidence of erectile dysfunction. Although improved nerve-sparing techniques have reduced the incidence of nerve injury, and the administration of phosphodiesterase type 5 inhibitors has revolutionized the treatment of erectile dysfunction, this problem remains a considerable challenge. In recent years, scientists have focused on brain-derived neurotrophic factor and vascular endothelial growth factor in the treatment of cavernous nerve injury in rat models. Results showed that both compounds were capable of enhancing the regeneration of the cavernous nerve and that activation of the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway played a major role in the process.
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INTRODUCTION

Radical prostatectomy in prostate cancer patients and other retroperitoneal surgical operations, such as radical rectal cancer surgeries, often lead to cavernous nerve damage, resulting in a high incidence of erectile dysfunction (ED); this has opened a new field of ED treatment. Many investigators have tried to develop novel methods of reducing the incidence of ED. Walsh and colleagues[1] developed a nerve-sparing technique to preserve potency after radical prostatectomy and cystoprostatectomy. However, in their study of 250 patients, sexual potency was impaired by 37% in patients who underwent bilateral nerve-sparing prostatectomy. Although the use of phosphodiesterase type 5 inhibitors has revolutionized the treatment of ED, this complication after surgery persists.[2] Many urologists focus on methods for reducing the likelihood of ED after surgery and accelerating regeneration of the cavernous nerve.

Although neuromodulatory therapy, which consists of neuroprotective and neurotrophic interventions, is based on the extremely limited intrinsic recovery ability of peripheral nerves, it has recently been proposed as the next breakthrough in cavernous nerve functional restoration.[3]

The neurotrophins, including brain-derived neurotrophic factor (BDNF) and nerve growth factor, comprise a large family of closely related proteins that are expressed in a variety of cell types. They were first identified as survival factors for sympathetic and sensory neurons and were subsequently found to have a wide range of influences on cellular activities, including transfer signals as well as neuronal differentiation, development and regeneration.[4,5] Neurotrophins regulate cell activities through PI-3 kinase (PI3K) cascades and a series of signalling pathways mediated by G proteins, such as Ras and Ras/Rho.[6] In the past decade, pioneering researchers have embarked on a series of explorations of the mechanism of cavernous nerve regeneration with neurotrophins, especially BDNF and vascular endothelial growth factor (VEGF); this led to the discovery of the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway, which played an important role in promoting neurite growth in the major pelvic ganglion (MPG) in rat models.[7]

The JAK/STAT pathway was first identified as the signal transduction pathway downstream of the cytokine receptors; these receptors, which are stimulated by cytokines, hormones, vasoactive agents and growth factors, lead to JAK phosphorylation and activation. Next, STAT binds to the activated receptors and is phosphorylated and translocated into the nucleus, where it acts as a transcription factor.[8] The JAK/STAT pathway plays a role in a large number of normal and abnormal physiological processes, including intestinal lineage differentiation,[9] human leukaemias and myeloproliferative disorders,[10] and endothelial cell migration and proliferation. Recently, there has been increasing evidence of an important role of the JAK/STAT pathway in peripheral nerve regeneration.[6,11,12]

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In this article, we focus on attempts to use BDNF and VEGF to promote the regeneration of the cavernous nerve after injury and examine the relationship between these growth factors and the JAK/STAT pathway.

BDNF AND VEGF

BDNF is a member of the mammalian family of neurotrophins, which also includes nerve growth factor and neurotrophins 3, 4 and 5. In 1982, Barde and colleagues[13] initially identified BDNF on the basis of its survival-promoting effect on cultured chick embryonic dorsal root ganglion neurons. Since then, additional BDNF activities have been discovered. BDNF could also promote neuronal survival during development and prevent neuronal death in experimental neuropathy models.[14,15] Recent studies have revealed that BDNF could provide neurotrophic support for a host of neuron types, such as peripheral sensory neurons and motor neurons.[13,16,17] Because of its wide range of activities, BDNF is being considered in the treatment of a variety of neurodegenerative diseases.[18]

BDNF promotes cell survival through two types of receptors: high-affinity tyrosine kinase B (TrkB) receptors and low-affinity pan-neurotrophin 75 (p75) receptors.[19] Binding of the Src homology domain-containing adaptor protein to TrkB leads to the recruitment of growth factor receptor-bound protein-2 and subsequent activation of Ras; this in turn activates the PI3K, mitogen-activated extracellular signal-regulated kinase (MEK1/2) and extracellular signal-regulated kinase (ERK1/2) pathways. Ribosomal S6 kinase phosphorylates cAMP response element-binding protein and other transcription factors. PI3K directly regulates certain survival pathways by activating serine/threonine protein kinase (Akt), which leads to neurogenesis. Another signalling pathway that is activated by TrkB involves phospholipase-Cγ, which results in the activation of protein kinase C *via* inositol 1,4,5-trisphosphate and diacylglycerol; this promotes long-term cellular responses related to proliferation and migration (Figure 1).

VEGF is an extensive endothelial cell-specific mitogen *in vitro* and is capable of inducing angiogenesis in a variety of *in vivo* models. Brown's study[20,21] showed that VEGF might have important and previously unsuspected roles in types of tissue other than the endothelium. Furthermore, Sondell *et al.*[22] revealed that VEGF acted as a neurotrophic factor and could stimulate axonal outgrowth through the flk-1 receptor (VEGF-R2). VEGF promotes nerve regeneration

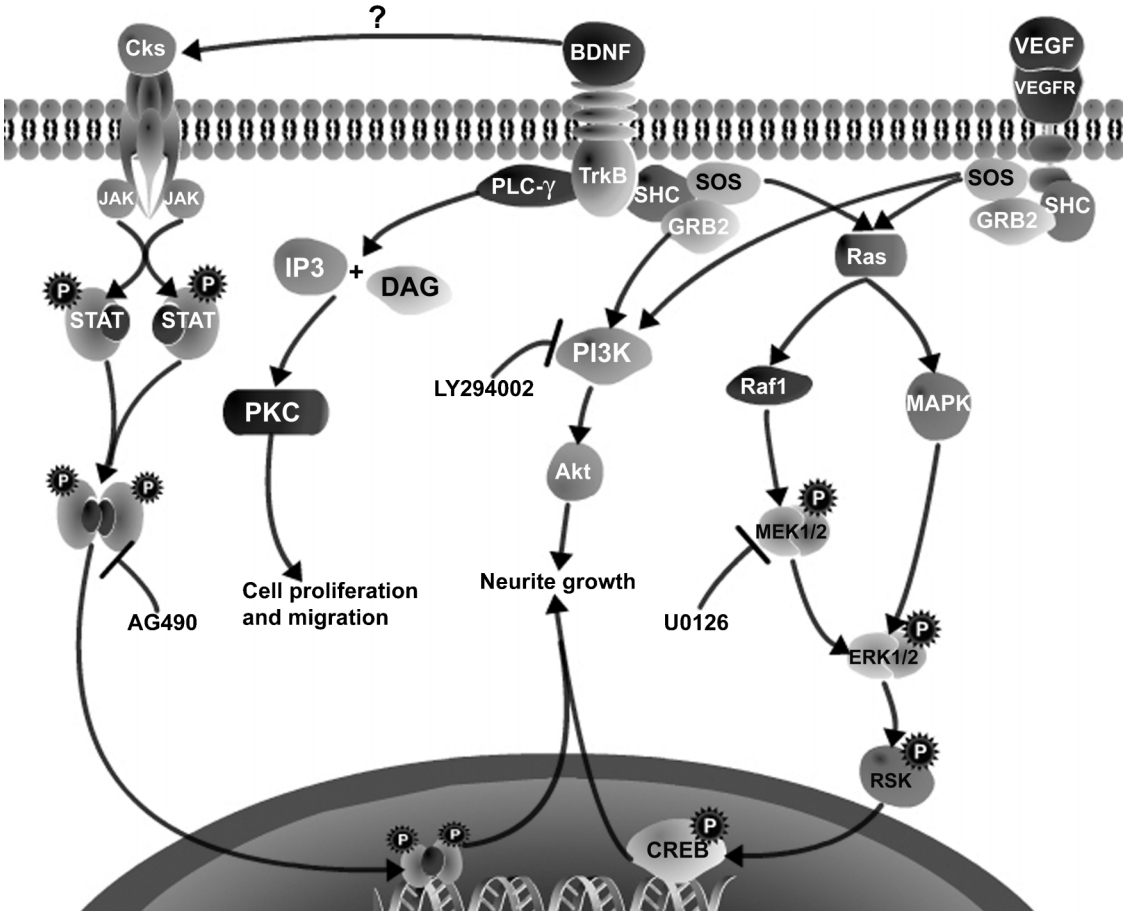

Figure 1 The activated TrkB receptor and the VEGF receptor recruit several specific small cytoplasmic signalling G proteins, including SHC, GRB2 and the Ras/Rho family; they are also involved in pathways regulated by MAPK, PI3K and ERK, evoking cellular responses that govern proliferation, migration and other processes. JAK activation occurs at cytokine-mediated receptors and subsequently results in the phosphorylation of STATs. To date, we have no evidence that BDNF and VEGF activate the JAK/STAT pathway by direct binding. Arrows indicate activation; T-shaped arrows indicate inhibition. Akt, serine/threonine protein kinase; BDNF, brain-derived neurotrophic factor; Cks, cytokines; CREB, cAMP response element-binding protein; DAG, diacylglycerol; ERK, extracellular signal-regulated kinase; GRB2, growth factor receptor-bound protein-2; IP3, inositol 1,4,5-trisphosphate; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated extracellular signal-regulated kinase; PKC, protein kinase C; PI3K, PI-3 kinase; PLC-γ, phospholipase-Cγ; RSK, ribosomal S6 kinase; STAT, signal transducer and activator of transcription; SHC, Src homology domain-containing adaptor protein; TrkB, tyrosine kinase B; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

mainly through the Ras/Raf pathway (Figure 1). However, some other reports have demonstrated that VEGF-induced neuroprotection is mediated through an flk-1 receptor-dependent activation of the PI3K/Akt signalling pathway;[23] because of the evidence of motor neuron regeneration,[24] a more plausible hypothesis is that the PI3K/Akt pathway plays a key role in VEGF-mediated neurite outgrowth.

The ability of BDNF to prevent degeneration of neurons located in the MPG that contain neuronal nitric oxide synthase (NOS), along with its ability to enhance the recovery of erectile function in rat models of bilateral cavernous nerve injury, has been demonstrated using BDNF gene therapy with adeno-associated virus-mediated production of neurotrophic factors.[25] Additionally, the ability of VEGF to induce the isoforms of NOS (endothelial NOS and inducible NOS) in the penis after intracavernosal injection has been reported in rat models of ligated pudendal arteries.[26] The authors found that endothelial NOS was expressed in the endothelium and probably mediated the angiogenic process; inducible NOS was expressed in smooth muscle and might provide protection against ischemic injury.[26] The lack of neuronal NOS indicated that it might play an incidental role in the angiogenic or injury responses. Later, researchers showed that BDNF could enhance the regeneration of neuronal NOS-containing nerve fibres.[27,28] In 2003, Lin *et al.*[12] first observed the function of VEGF and BDNF in enhancing the outgrowth of nerve fibres on cultured MPG *in vitro* in serum-free medium supplemented with VEGF (50 ng ml^{-1}), BDNF, and neurotrophins 3 and 4 (each at a concentration of 20 ng ml^{-1}). After 2 days of culture, the specimens were stained for NOS, tyrosine hydroxylase and acetylcholinesterase. Lin *et al.*[12] found that BDNF and VEGF were both capable of inducing fibres that express NOS, tyrosine hydroxylase and acetylcholinesterase.

Lee and colleagues[29] injected VEGF intracavernously to test its erectile restoration effect in rat models of traumatic arteriogenic ED. They ligated both internal iliac arteries in rats to create traumatic arteriogenic ED models and then injected VEGF (2 μg versus 4 μg) into the cavernosum. The results showed that VEGF treatment facilitated the recovery of erectile function compared with the sham-operated group. The recovery in the 4-μg group was more pronounced than in the 2-μg group. Recovery of neuronal NOS-positive nerve fibres was also noted after VEGF treatment. The responsible mechanism might involve the ability of VEGF to promote neovascularisation in ischemic tissues and possibly to exert neurotrophic effects.[12]

Many study groups have made remarkable contributions to other clinical applications. The retrograde transport of BDNF from axons to the neuronal cell body provided a theoretical basis for many of these studies.[30] One group tried a new approach in which bilateral nerves were crushed with a haemostat for 2 min and then intracavernously injected with BDNF (600 ng/rat) and VEGF (4000 ng/rat) to investigate their function in nerve regeneration and recovery of erectile function.[31] They later suggested that the optimal dose of both factors for promoting MPG neurite growth was 25–50 ng ml^{-1} in the *in vitro* system.[32] Neurite growth from aged rats were not as robust as growth from tissue from younger rats, which indicates that future clinical trials might reveal important differences in the responsiveness of aged human tissue to these types of treatments.[32]

JAK/STAT PATHWAY

Research on interferons has found that they transduce signals through a new type of signalling pathway that involves activation of transcription factors at the cell membrane, now known as STATs. STATs are activated by the JAK family of protein tyrosine kinases. Subsequent studies identified four JAKs (Jak1, Jak2, Jak3 and Tyk2)[33–36] and seven STATs[37–44] (STATs 1, 2, 3, 4, 5a, 5b and 6), which are involved in a different signal transduction pathway mechanism. In mammals, the JAK/STAT pathway is becoming increasingly known as the principal signalling mechanism for a wide range of growth factors and cytokines;[45,46] it plays an important role in cell proliferation, differentiation, migration and apoptosis. Any mutation of JAK/STAT will affect the activity of the pathway.[47,48]

In recent years, researchers have found that activation of the JAK/STAT pathway can enhance the process of peripheral nerve regeneration.[6,11,12] STAT3 activation is central to the enhanced ability of neurons to grow and is implicated in accelerated axonal regeneration.[11] It has been shown that phosphorylation of STAT3 confers the ability to translocate into the nucleus, where it combines with genomic regulatory sequences and enhances the transcription of related genes.[49] Only a few genes have thus far been defined as targets of STAT3, including neuropeptide vasoactive intestinal peptide and c-Jun.[50–53] The results of a study by Sheu *et al.*[54] suggested that the activation of the JAK/STAT3 pathway might be related to axonal regeneration and the proliferation and migration of Schwann cells after sciatic nerve injury. After transection of cavernous nerves in rat models, the phosphorylation levels of STAT3 and STAT1 also increased significantly as compared with a control group ($P<0.01$).[55]

As mentioned above, BDNF promotes neuronal survival and neurite outgrowth and prevents neuronal degeneration; most of the mechanisms and the specific signalling pathways remained unclear although some have already been identified, including Ras and cdc-42/ras/rho G protein families as well as the mitogen-activated protein kinase (MAPK), PI3K and phospholipase-Cγ cascades. Although BDNF transfers signals into cells through the TrkB and p75 receptors, some reports showed no role for MAPK/ERK and PI3K in mature peripheral neuron regeneration.[56] Studies by Gallo *et al.*[57] and Liu and Snider[58] showed that maintenance of the protective effects of BDNF was independent of protein kinase A activity and a JAK2 inhibitor prevented the regenerative axonal response to a conditional lesion; this suggests the existence of an alternative JAK/STAT link between cytokine signalling and axon growth. To explore the mechanism of the neurite growth enhancement of explant MPG cultures, researchers[7] tried inhibitors of four candidate signalling pathways—MEK (U0126), PI3K (LY294002), protein kinase A (KT5720) and JAK/STAT (AG490)—supplemented with BDNF. The investigators found that AG490 significantly reduced BDNF-enhanced neurite growth as compared with the other pathways, which suggests that BDNF acts primarily *via* the JAK/STAT pathway to promote neurite growth in the rat MPG; they confirmed the findings in another study.[6] The researchers proposed that BDNF exerted its effect *via* the induction of phosphorylation of JAK2, STAT1 and STAT3. Increased expression of penile BDNF and upregulation of phosphorylated STAT1 and STAT3 in MPG were observed directly in response to cavernous nerve transection,[55] which provided better support for the theories described above. However, we do not yet know whether JAK/STAT is directly activated by BDNF. A more likely mechanism involves a secondary procedure; that is, Schwann cells are activated by BDNF and then modulate the local environment to enhance the regeneration process *via* the synthesis and release of surface cell adhesion molecules, extracellular matrix proteins and neurotrophic factors, which will ultimately activate the JAK/STAT pathway.[59] The potential role of Schwann cells in BDNF-mediated neurite outgrowth requires further investigation.

With regard to the interaction between the VEGF and JAK/STAT pathways, most studies have focused on their relationship to

tumours[60,61] or cellular invasiveness.[62] As yet, there is no evidence that supports a role for VEGF in promoting neurite outgrowth by directly activating the JAK/STAT pathway. VEGF may achieve its goal through its angiogenesis effect or by activating the JAK/STAT pathway by direct binding in a manner similar to that of the other neurotrophic factors. However, evidence that STAT3 activation elevates VEGF expression levels[62] could provide another possibility. The increased VEGF expression after cavernous nerve injury induced by elevated STAT3 phosphorylation levels[55] may further activate the JAK/STAT pathway. The VEGF and JAK/STAT pathways directly or indirectly interact with each other.

Despite abundant emerging evidence that the JAK/STAT pathway plays a central role in cavernous nerve regeneration after injury, the details of the mechanism remain unknown. The potential mechanisms and their overall effects on nerve regeneration require further investigation. Therapeutic advances will be developed when a better understanding of the JAK/STAT pathway is achieved.

CONCLUSION

VEGF and BDNF are both capable of enhancing regeneration of the cavernous nerve after injury. The JAK/STAT signalling pathway plays an important role in this process, and, although the mechanism needs to be elucidated in detail, it clearly represents a promising new molecular target for modulating nerve survival and regeneration.

AUTHOR CONTRIBUTIONS

HYZ and TFL contributed the study concept and design; HYZ wrote the draft of the manuscript under the supervision of XBJ and TFL, who also performed critical revision of it.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1 Catalona WJ, Bigg SW. Nerve-sparing radical prostatectomy: evaluation of results after 250 patients. *J Urol* 1990; **143**: 538–43; discussion 44.
2 Bella AJ, Deyoung LX, Al-Numi M, Brock GB. Daily administration of phosphodiesterase type 5 inhibitors for urological and nonurological indications. *Eur Urol* 2007; **52**: 990–1005.
3 Burnett AL, Lue TF. Neuromodulatory therapy to improve erectile function recovery outcomes after pelvic surgery. *J Urol* 2006; **176**: 882–7.
4 Ernsberger U. Role of neurotrophin signalling in the differentiation of neurons from dorsal root ganglia and sympathetic ganglia. *Cell Tissue Res* 2009; **336**: 349–84.
5 Fiore M, Chaldakov GN, Aloe L. Nerve growth factor as a signaling molecule for nerve cells and also for the neuroendocrine-immune systems. *Rev Neurosci* 2009; **20**: 133–45.
6 Lin G, Bella AJ, Lue TF, Lin CS. Brain-derived neurotrophic factor (BDNF) acts primarily *via* the JAK/STAT pathway to promote neurite growth in the major pelvic ganglion of the rat: part 2. *J Sex Med* 2006; **3**: 821–7; discussion 8–9.
7 Bella AJ, Lin G, Tantiwongse K, Garcia M, Lin CS *et al*. Brain-derived neurotrophic factor (BDNF) acts primarily *via* the JAK/STAT pathway to promote neurite growth in the major pelvic ganglion of the rat: part I. *J Sex Med* 2006; **3**: 815–20.
8 Lai SY, Johnson FM. Defining the role of the JAK-STAT pathway in head and neck and thoracic malignancies: implications for future therapeutic approaches. *Drug Resist Updat* 2010; **13**: 67–78.
9 Lin G, Xu N, Xi R. Paracrine unpaired signaling through the JAK/STAT pathway controls self-renewal and lineage differentiation of drosophila intestinal stem cells. *J Mol Cell Biol* 2009; **2**: 37–49.
10 Tefferi A. Classification, diagnosis and management of myeloproliferative disorders in the JAK2V617F era. *Hematology Am Soc Hematol Educ Program* 2006: 240–5.
11 Xu JJ, Chen EY, Lu CL, He C. Recombinant ciliary neurotrophic factor promotes nerve regeneration and induces gene expression in silicon tube-bridged transected sciatic nerves in adult rats. *J Clin Neurosci* 2009; **16**: 812–7.
12 Lin G, Chen KC, Hsieh PS, Yeh CH, Lue TF *et al*. Neurotrophic effects of vascular endothelial growth factor and neurotrophins on cultured major pelvic ganglia. *BJU Int* 2003; **92**: 631–5.
13 Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. *EMBO J* 1982; **1**: 549–53.
14 Yan Q, Rosenfeld RD, Matheson CR, Hawkins N, Lopez OT *et al*. Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. *Neuroscience* 1997; **78**: 431–48.
15 Frostick SP, Yin Q, Kemp GJ. Schwann cells, neurotrophic factors, and peripheral nerve regeneration. *Microsurgery* 1998; **18**: 397–405.
16 Oppenheim RW, Yin QW, Prevette D, Yan Q. Brain-derived neurotrophic factor rescues developing avian motoneurons from cell death. *Nature* 1992; **360**: 755–7.
17 Sendtner M, Holtmann B, Kolbeck R, Thoenen H, Barde YA. Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. *Nature* 1992; **360**: 757–9.
18 Apfel SC. Neurotrophic factors in peripheral neuropathies: therapeutic implications. *Brain Pathol* 1999; **9**: 393–413.
19 Bariohay B, Lebrun B, Moyse E, Jean A. Brain-derived neurotrophic factor plays a role as an anorexigenic factor in the dorsal vagal complex. *Endocrinology* 2005; **146**: 5612–20.
20 Rahbar R, Brown LF, Folkman J, McGill TJ, Healy GB *et al*. Role of vascular endothelial growth factor A in children with acquired airway stenosis. *Ann Otol Rhinol Laryngol* 2007; **116**: 430–5.
21 Arbiser JL, Johnson D, Cohen C, Brown LF. High-level expression of vascular endothelial growth factor and its receptors in an aphthous ulcer. *J Cutan Med Surg* 2003; **7**: 225–8.
22 Sondell M, Sundler F, Kanje M. Vascular endothelial growth factor is a neurotrophic factor which stimulates axonal outgrowth through the flk-1 receptor. *Eur J Neurosci* 2000; **12**: 4243–54.
23 Jin KL, Mao XO, Greenberg DA. Vascular endothelial growth factor rescues HN33 neural cells from death induced by serum withdrawal. *J Mol Neurosci* 2000; **14**: 197–203.
24 Kermer P, Klocker N, Labes M, Bahr M. Insulin-like growth factor-I protects axotomized rat retinal ganglion cells from secondary death *via* PI3-K-dependent Akt phosphorylation and inhibition of caspase-3 *in vivo*. *J Neurosci* 2000; **20**: 2–8.
25 Bakircioglu ME, Lin CS, Fan P, Sievert KD, Kan YW *et al*. The effect of adeno-associated virus mediated brain derived neurotrophic factor in an animal model of neurogenic impotence. *J Urol* 2001; **165**: 2103–9.
26 Lin CS, Ho HC, Chen KC, Lin G, Nunes L *et al*. Intracavernosal injection of vascular endothelial growth factor induces nitric oxide synthase isoforms. *BJU Int* 2002; **89**: 955–60.
27 Chen KC, Minor TX, Rahman NU, Ho HC, Nunes L *et al*. The additive erectile recovery effect of brain-derived neurotrophic factor combined with vascular endothelial growth factor in a rat model of neurogenic impotence. *BJU Int* 2005; **95**: 1077–80.
28 Mitsui T, Fischer I, Shumsky JS, Murray M. Transplants of fibroblasts expressing BDNF and NT-3 promote recovery of bladder and hindlimb function following spinal contusion injury in rats. *Exp Neurol* 2005; **194**: 410–31.
29 Lee MC, El-Sakka AI, Graziottin TM, Ho HC, Lin CS *et al*. The effect of vascular endothelial growth factor on a rat model of traumatic arteriogenic erectile dysfunction. *J Urol* 2002; **167**: 761–7.
30 Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci* 2001; **24**: 677–736.
31 Hsieh PS, Bochinski DJ, Lin GT, Nunes L, Lin CS *et al*. The effect of vascular endothelial growth factor and brain-derived neurotrophic factor on cavernosal nerve regeneration in a nerve-crush rat model. *BJU Int* 2003; **92**: 470–5.
32 Lin G, Shindel AW, Fandel TM, Bella AJ, Lin CS *et al*. Neurotrophic effects of brain-derived neurotrophic factor and vascular endothelial growth factor in major pelvic ganglia of young and aged rats. *BJU Int* 2009; **105**: 114–20.
33 Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW. Signaling through the JAK/STAT pathway, recent advances and future challenges. *Gene* 2002; **285**: 1–24.
34 Levy DE, Darnell JE Jr. Stats: transcriptional control and biological impact. *Nat Rev* 2002; **3**: 651–62.
35 O'Shea JJ, Gadina M, Schreiber RD. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. *Cell* 2002; **109** Suppl: S121–31.
36 Wilks AF. Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction. *Proc Natl Acad Sci USA* 1989; **86**: 1603–7.
37 Yamamoto K, Quelle FW, Thierfelder WE, Kreider BL, Gilbert DJ *et al*. Stat4, a novel gamma interferon activation site-binding protein expressed in early myeloid differentiation. *Mol Cell Biol* 1994; **14**: 4342–9.
38 Hou J, Schindler U, Henzel WJ, Ho TC, Brasseur M *et al*. An interleukin-4-induced transcription factor: IL-4 Stat. *Science* 1994; **265**: 1701–6.
39 Quelle FW, Shimoda K, Thierfelder W, Fischer C, Kim A *et al*. Cloning of murine Stat6 and human Stat6, Stat proteins that are tyrosine phosphorylated in responses to IL-4 and IL-3 but are not required for mitogenesis. *Mol Cell Biol* 1995; **15**: 3336–43.
40 Akira S, Nishio Y, Inoue M, Wang XJ, Wei S *et al*. Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. *Cell* 1994; **77**: 63–71.
41 Zhong Z, Wen Z, Darnell JE Jr. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science* 1994; **264**: 95–8.
42 Zhong Z, Wen Z, Darnell JE Jr. Stat3 and Stat4: members of the family of signal transducers and activators of transcription. *Proc Natl Acad Sci USA* 1994; **91**: 4806–10.
43 Jacobson NG, Szabo SJ, Weber-Nordt RM, Zhong Z, Schreiber RD *et al*. Interleukin 12 signaling in T helper type 1 (Th1) cells involves tyrosine phosphorylation of signal transducer and activator of transcription (Stat)3 and Stat4. *J Exp Med* 1995; **181**: 1755–62.

44 Wakao H, Gouilleux F, Groner B. Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. *EMBO J* 1994; **13**: 2182–91.
45 Darnell JE, Jr. Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 1994; **264**: 1415–21.
46 Ihle JN, Kerr IM. Jaks and Stats in signaling by the cytokine receptor superfamily. *Trends Genet* 1995; **11**: 69–74.
47 Meyer T, Begitt A, Lodige I, van Rossum M, Vinkemeier U. Constitutive and IFN-gamma-induced nuclear import of STAT1 proceed through independent pathways. *EMBO J* 2002; **21**: 344–54.
48 Melen K, Kinnunen L, Julkunen I. Arginine/lysine-rich structural element is involved in interferon-induced nuclear import of STATs. *J Biol Chem* 2001; **276**: 16447–55.
49 Ip NY, Yancopoulos GD. The neurotrophins and CNTF: two families of collaborative neurotrophic factors. *Annu Rev Neurosci* 1996; **19**: 491–515.
50 Symes A, Gearan T, Eby J, Fink JS. Integration of Jak-Stat and AP-1 signaling pathways at the vasoactive intestinal peptide cytokine response element regulates ciliary neurotrophic factor-dependent transcription. *J Biol Chem* 1997; **272**: 9648–54.
51 Symes A, Lewis S, Corpus L, Rajan P, Hyman SE *et al*. STAT proteins participate in the regulation of the vasoactive intestinal peptide gene by the ciliary neurotrophic factor family of cytokines. *Mol Endocrinol* 1994; **8**: 1750–63.
52 Coffer P, Lutticken C, van Puijenbroek A, Klop-de Jonge M, Horn F *et al*. Transcriptional regulation of the junB promoter: analysis of STAT-mediated signal transduction. *Oncogene* 1995; **10**: 985–94.
53 Schaefer TS, Sanders LK, Nathans D. Cooperative transcriptional activity of Jun and Stat3 beta, a short form of Stat3. *Proc Natl Acad Sci USA* 1995; **92**: 9097–101.
54 Sheu JY, Kulhanek DJ, Eckenstein FP. Differential patterns of ERK and STAT3 phosphorylation after sciatic nerve transection in the rat. *Exp Neurol* 2000; **166**: 392–402.
55 Bella AJ, Lin G, Garcia MM, Tantiwongse K, Brant Wo *et al*. Upregulation of penile brain-derived neurotrophic factor (BDNF) and activation of the JAK/STAT signalling pathway in the major pelvic ganglion of the rat after cavernous nerve transection. *Eur Urol* 2007; **52**: 574–81.
56 Kaplan DR, Miller FD. Neurotrophin signal transduction in the nervous system. *Curr Opin Neurobiol* 2000; **10**: 381–91.
57 Gallo G, Ernst AF, McLoon SC, Letourneau PC. Transient PKA activity is required for initiation but not maintenance of BDNF-mediated protection from nitric oxide-induced growth-cone collapse. *J Neurosci* 2002; **22**: 5016–23.
58 Liu RY, Snider WD. Different signaling pathways mediate regenerative versus developmental sensory axon growth. *J Neurosci* 2001; **21**: RC164.
59 Ogata T, Yamamoto S, Nakamura K, Tanaka S. Signaling axis in schwann cell proliferation and differentiation. *Mol Neurobiol* 2006; **33**: 51–62.
60 Chen H, Ye D, Xie X, Chen B, Lu W. VEGF, VEGFRs expressions and activated STATs in ovarian epithelial carcinoma. *Gynecol Oncol* 2004; **94**: 630–5.
61 Roorda BD, Ter Elst A, Scherpen FJ, Meeuwsen-de Boer TG, Kamps WA *et al*. VEGF-A promotes lymphoma tumour growth by activation of STAT proteins and inhibition of p27(KIP1) *via* paracrine mechanisms. *Eur J Cancer* 2010; **46**: 974–82.
62 Wang Z, Luo F, Li L, Yang L, Hu D *et al*. STAT3 activation induced by Epstein-Barr virus latent membrane protein1 causes vascular endothelial growth factor expression and cellular invasiveness *via* JAK3 and ERK signaling. *Eur J Cancer* 2010; **46**: 2996–3006.