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Pleiotropic Role of VEGF and Its Application for Traumatic Spinal Cord Injury

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Abstract

Traumatic Spinal Cord Injury (SCI) is comprised of an initial mechanical insult to the spinal cord, followed by a secondary wave of injury, resulting in a toxic lesion environment which damages surrounding neurons, axons and glial cells. The minimal axonal growth in the Central Nervous System (CNS) including the spinal cord following injury is in stark contrast to the Peripheral Nervous System (PNS), which demonstrates endogenous axonal regeneration and repair. This review focuses on the pleiotropic effects of Vascular Endothelial Growth Factor (VEGF) on neurons and various types of glial cells, with a brief discussion of its well-characterized canonical role in the cardiovascular system and cancer. Recent decades of studies strongly suggest that combinational treatment approaches hold the greatest therapeutic potential for CNS trauma. Therefore, future directions of combinational therapies will be also proposed.

Keywords: Spinal cord injury; Vascular endothelial growth factor; Angiogenesis; Placental growth factor; Neuropilin; Combinational therapies; Neuro trauma

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SCI Background and Need for Therapies

Spinal Cord Injury (SCI) is debilitating and devitalizing, and currently no effective treatments exist. Based upon a thorough and systematic review of global statistics, starting from 5,874 articles with a final inclusion of 48 articles [1] recently reported the worldwide SCI cases, with the United States having the highest prevalence (906 cases per 1 million people). New Zealand had the highest national incidence (49.1 cases of SCI per 1 million people), while Spain (8 cases of SCI per 1 million people) and Fiji (10 cases of SCI per 1 million people) had the lowest national incidence. Motor vehicle accidents are the primary cause of SCI cases worldwide, with falls and sports injuries typically being second and third for most countries [1]. In addition to the long-term potential of chronic pain, inflammation, and devastating disabilities that SCI patients endure, the lifetime cost of one patient is approximately 1-4.5 million United States dollars, depending upon the patient's age and level of injury (Christopher Reeve Foundation website, NSCISC – National Spinal Cord Injury Statistical Center). It is estimated that the national cost in the United States is more than $400 billion US dollars for current and future healthcare for SCI patients. The initial trauma resulting from SCI disrupts local vasculature leading to blood-spinal cord barrier breakdown [2-5] which is followed by secondary damage involving hemorrhage, ischemia [6], excitotoxicity, edema, and sustained chronic inflammation [7], leading to neuronal death, axonal degeneration, and glial scar formation. Disruption of local vasculature likely leads to downstream neuronal apoptosis, axonal die-back, and loss of gray and white matter tissue [8]. Despite the toxic milieu that exists after SCI, an endogenous angiogenic response occurs that peaks between 7-14 days post-injury [9,10], and then regresses coinciding with the formation of cystic cavitation in both rats and higher primates. Popovich et al. described the high revascularization plasticity of the spinal cord vasculature even up to 28 days post SCI [5]. Together, these previous findings display the potential therapeutic target of the vasculature, a time window for treatment, and the need for a growth-permissive tissue scaffold within the lesion, to provide a structural matrix for the remodeling vasculature.

Vascular Endothelial Growth Factor (VEGF) is an important signaling molecule intimately associated with angiogenesis [11,12], axonal guidance [13,14], neuroprotection [16-19], Schwann cell survival and migration, and proliferation of astrocytes, microglia, and neural stem cells [16]. Thus, making this pro-angiogenic factor a therapeutic agent for promoting spinal cord revascularization, neuroprotection, cell proliferation, tissue regeneration, and ultimately improved functional recovery. This review, therefore, focuses on the background of VEGF as an angiogenic trophic factor and its more recently discovered pleiotropic role in the nervous systems, as well as its

Table 1: Cytogenetic location of VEGF family proteins.

Protein	Cytogenetic location	References
VEGF-A	cytogenetic location 6p12, 9 exons	[31]
VEGF-B	cytogenetic location 11q13, 7 exons	[32]
VEGF-C	cytogenetic location 4q34.3, 7 exons	[33]
VEGF-D	cytogenetic location Xp22.31, 7 exons	[34]
VEGF-E	Orf virus, cytogenetic location 4q32, 8 exons	[36]
PGF	cytogenetic location 14q24.3, 7 exons	[38]
VEGF Receptor 1	cytogenetic location 13q12, 33 exons	[41]
VEGF Receptor 2	cytogenetic location 4q11-q12, 30 exons	[42]
VEGF Receptor 3	cytogenetic location 5q35.3, 34 exons	[43]

potential influence for tissue repair following traumatic spinal cord injury.

Discovery of VEGF and Its Receptors

Vascular Endothelial Growth Factor (VEGF) is well known for its influence on vasculature, and has been widely studied and characterized in cardiovascular and cancer research and medicine. In more recent decades, VEGF has also been recognized for its role in embryonic development [1], its pleiotropic effects [14,16,20] on neurons and glial, and its therapeutic potential to prevent neurodegeneration [21-24].

VEGF-A (Vascular endothelial growth factor A) binds to VEGFR-1 (VEGF Receptor 1), VEGFR-2 (VEGF Receptor 2), NRP-1 (Neuropilin-1 receptor) and NRP-2 (Neuropilin-2 receptor). VEGF-B (Vascular endothelial growth factor B) and PGF (Placental Growth Factor) bind to VEGFR-1. VEGF-C (Vascular endothelial growth factor C) and VEGF-D (Vascular endothelial growth factor D) bind to VEGFR-2 (VEGF Receptor 2) and VEGFR-3 (VEGF Receptor 3). Downstream signaling leads to angiogenesis, vasculogenesis, lymphanogiogenesis, vascular permeability, cell survival (inhibition of apoptosis), migration, proliferation, and mobilization of progenitors. Abbreviations: VEGF-A (Vascular Endothelial Growth Factor A), VEGF-B (Vascular Endothelial Growth Factor B), VEGF-C (Vascular Endothelial Growth Factor C), VEGF-D (Vascular Endothelial Growth Factor D), PGF (Placental Growth Factor), VEGFR-1 (Vascular Endothelial Growth Factor Receptor 1), VEGFR-2 (Vascular Endothelial Growth Factor Receptor 2), VEGFR-3 (Vascular Endothelial Growth Factor Receptor 3), NRP-1 (Neuropilin-1 Receptor), NRP-2 (Neuropilin-2 Receptor), PI3K (Phosphatidylinositol-4,5-bisphosphate 3-kinase), Rac (Ras-related C3 botulinum toxin substrate 1), Ras (Rat sarcomas, small GTPase), RhoA (Ras homolog gene family, member A), FAK (Focal Adhesion Kinase), PTEN (Phosphatase and tensin homolog), Paxillin, Survivin, Caspase-9, Akt (Protein kinase B), FOX (Forkhead box), PLC-γ (Phospholipase C, gamma), PKC (Protein kinase C), BAD (Bcl-2-associated death promoter), Raf (Rapidly Accelerated Fibrosarcoma), mTOR (mammalian target of rapamycin), ROC (Ras of Complex protein), NO (Nitric oxide), eNOS (endothelial Nitric Oxide Synthase), AA (Arachidonic acid), cPLA2 (calcium-dependent Phospholipase A2), ERK (Extracellular signal Regulated Kinases), MEK (Mitogen-activated protein kinase).

Dr. Judah Folkman and colleagues extensively studied endothelial cell activation and angiogenesis, intricately associated with tumor growth and survival, characterizing the trophic factor as Tumor-Angiogenesis Factor (TAF) [11] with seminal papers during the 1970s. Previous studies displayed the factor's potential to act at a distance, after diffusing across a membrane [25,26]. VEGF, a gene family comprised of five major proteins along with receptors and co-receptors, was originally termed Vascular Permeability Factor (VPF) in 1983 [27] by Drs. Sanger and Dvorak. In 1989, this vascular trophic factor, which resulted in extensive endothelial cell outgrowth and angiogenesis, was termed VEGF by Drs. Ferrara and Henzel at Genentech [28]. The neurovascular evolution of VEGF and its influence on the nervous system was summarized by Zacchigna, Carmeliet and colleagues, displaying the importance of the human VEGF homologue in *C. elegans* (*Caenorhabditis elegans*) and Drosophila melanogaster, which lack blood vessels or have very few, respectively [19]. Additionally, Popovici et al. described receptors on *C. elegans* neurons with structural similarity to the human VEGF receptors (VEGFR's) [29], which can activate human VEGFR's [19]. Dr. Peter Carmeliet has been instrumental in expanding and detailing the Belgian Anatomist, Andreas Vesalius' 1543 observations about the overlap of the nervous and vascular systems (*De humani corporis fabrica*, *On the fabric of the human body*, 1543), thus, displaying the pleiotropic influence of VEGF on the nervous, vascular, and immune systems.

Isoforms and Co-Receptors

The VEGF sub-family of growth factors belongs to the Platelet-Derived Growth Factor (PDGF) family, and is comprised of isoforms VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and PGF (placental growth factor), nicely summarized by Grünewald et al. [30] (Figure 1). Together, these trophic factors are responsible for embryonic vasculogenesis, the formation of new blood vessels, as well as the development of blood vessels from existing blood vessels (angiogenesis). Human VEGF exists as a homodimer (~45 kDa under non-reducing conditions, and ~23 kDa under reducing conditions; [28]) and is synonymous with VEGF-A. Alternative splicing of the human VEGF-A gene (cytogenetic location 6p12, 9 exons; [31]) generates five distinct VEGF-A monomers including $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$, designated by the number of amino acids in the sequence. VEGF-B is located on chromosome 11 (cytogenetic location 11q13, 7 exons; [32]). VEGF-C is located on chromosome 4 (cytogenetic location 4q34.3, 7 exons; [33]). VEGF-D is located on the X chromosome (cytogenetic location Xp22.31, 7 exons; [34]). The non-human VEGF-E, encoded by the parapoxvirus Orf virus [35] is located on chromosome 4 (cytogenetic location 4q32, 8 exons; [36]). PGF is located on chromosome 14 (cytogenetic location 14q24.3, 7 exons; [37]). VEGF-E binds selectively to VEGFR-2, and has been shown to promote lesion angiogenesis in response to the viral infection by the parapoxvirus Orf virus [35], and regulation of keratinocytes for wound re-epithelialization in response to the purified VEGF-E protein[15] that this virus encodes, thus making it a potentially good candidate for wound healing and repair. Moreover, since VEGF-E binds only to VEGFR-2 and not to VEGFR-1, it does not result in vascular permeability or tissue inflammation, like VEGF-A [36]. Thus, purified VEGF-E might have future potential in tissue repair beyond just would heal.

Two tyrosine kinase receptors, VEGF Receptor 1 (VEGFR-1, fms-like tyrosine kinase 1/Flt-1; [38]) and VEGF Receptor 2 (VEGFR-2, fetal liver kinase 1/Flk-1, kinase insert domain receptor/KDR) were identified in 1992 [39,40]. Neuropilin (NP1 and NP-2) co-receptors bind specifically to VEGF isoforms 165 ($VEGF_{165}$ in humans, $VEGF_{164}$ in rats). The human VEGFR-1 gene is located on chromosome 13

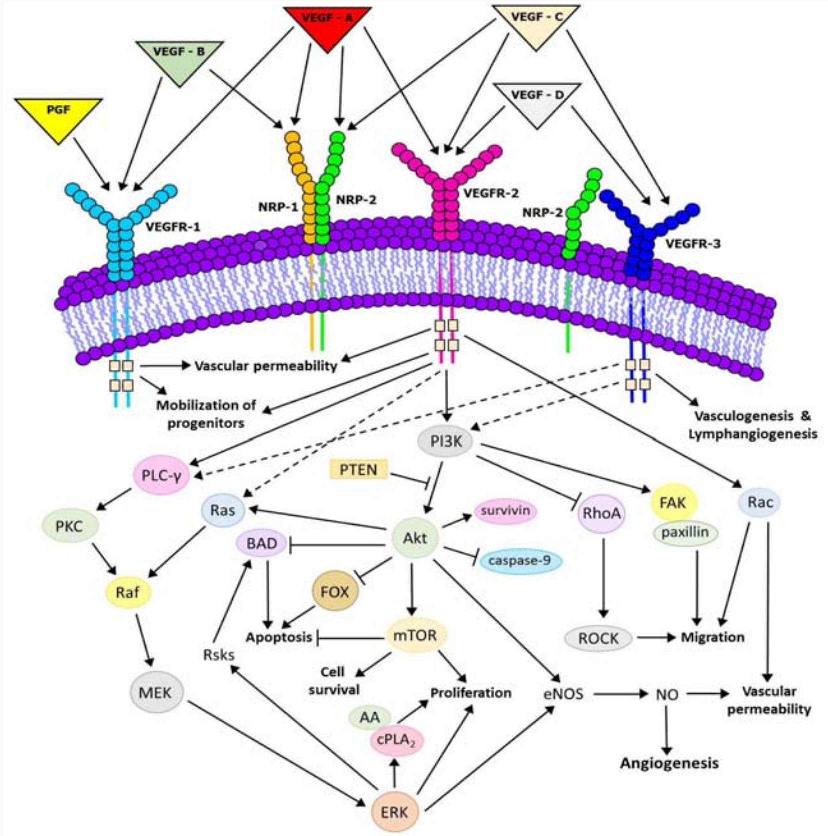

Figure 1: Intracellular signaling of VEGF.

(cytogenetic location 13q12, 32 exons; [41]) and VEGFR-2 gene is located on chromosome 4 (cytogenetic location 4q11-q12, 30 exons; [42]). VEGF Receptor 3 (VEGFR-3, fms-like tyrosine kinase 4/Flt-4; [43]) was independently characterized in 1992 by Galland et al. and Pajusola and colleagues [44,45]. All three VEGF Receptors are type V Receptor Tyrosine Kinases (RTK's), consisting of an extracellular region (7 immunoglobulin-like domains), a single transmembrane domain, a juxtamembrane component, and an intracellular protein-tyrosine kinase segment with a variable (70-100 amino acids) kinase insert and a carboxyterminal tail [46]. The main pathway promoting angiogenesis is the interaction of VEGF-A (VEGF) and its VEGFR-2 receptor; particularly, the phosphorylation of the VEGFR-2 Tyrosine residue 1175, which binds to the SH2-domain of Phospholipase-Cɣ (PLCɣ), upstream of the PKC mitogen-activated protein kinase/ extracellular signal-related kinases (MAPK/ERK) pathway. Ji et al. showed that PLCɣ knockout mice were embryonic lethal at approximately day E9 [47]. VEGFR-1 knockout mice were shown to be embryonic lethal at E8.5, resulting from disorganized vasculature and endothelial cell-overgrowth [48]; this study also displays the importance of the transmembrane domain of VEGFR-1, which localizes VEGF for signaling during embryogenesis, and negatively regulates angiogenesis. Takashima et al. observed embryonic lethality (E8.5) in NRP-1 and NRP-2 knockout animals, due to lack of blood vessel formation [49]. In 1996, both Carmeliet et al. and Ferrara et al. discovered the dose-dependent embryonic lethality of homozygous VEGF$^{-/-}$ knockout animals (E10.5 and E11-12, respectively) and heterozygous VEGF$^{+/-}$ animals (approximately E12.5), due to lack of formation of functional vasculature and significant cell apoptosis [12,50]. Furthermore, Ferrara et al. detailed the significantly diminished capacity for tumorigenesis of VEGF$^{-/-}$ knockout embryonic stem cells; thus, underscoring VEGF's role in tumor formation and the critical role of angiogenesis in tumor growth [50]. While VEGF, VEGFR-1 (Flt-1), and VEGFR-2 (Flk-1) are all essential components of embryonic development, these studies [12,50] highlight VEGF as the most vital factor, due to VEGF$^{+/-}$ embryonic lethality [50]. Collectively, these studies display the importance of VEGF, its receptors and downstream signaling pathways for angiogenesis, embryonic development, and tumorigenesis. VEGF ligand isoforms and receptor interactions are summarized in Figure 1.

Localization of VEGF and Its Receptors

Cytogenetic and tissue localization of VEGF and its receptors is summarized in Table 1 and Table 2, respectively. VEGF-A mRNA is widely expressed throughout the body, with the highest expression in the lungs, heart, adrenal glands, and kidneys, and lower expression in the liver, spleen, and gastric mucosa [46]. VEGF-A is also a major target for anti-tumor therapies, as VEGF-A is expressed by the following human tumors: colorectal, breast, non-small cell lung, and prostate [46]. VEGF-B is highly expressed in the heart, brain, testes, and kidney, with lower expression in spleen, lung, and liver [46]. VEGF-C is expressed in the heart, intestine, ovaries, and the placenta (HPRD: 03317; ID: 01889). VEGF-D is expressed in the colon, heart, kidney, liver, lung, ovaries, pancreas, prostate, skeletal muscles, small intestine, spleen and testis (HPRD: 02102, ID: 03237). PGF is expressed in the dentine matrix, endometrium, eyes, natural killer cells, placenta, serum, trophoblasts, umbilical vein endothelial cells, and vascular endothelium (HPRD: 03076, ID: 02102). VEGFR-1 is expressed in blood vessels, bone marrow, colon, endometrium,

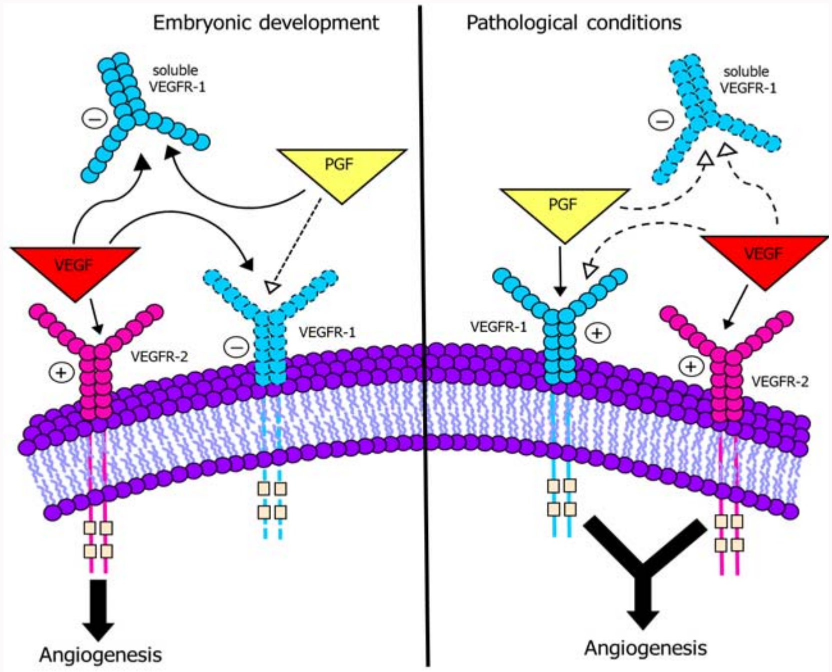

Figure 2: Pathological switch promoting angiogenesis: Synergistic activation of VEGFR-1 (soluble and membrane-bound forms) under embryonic conditions, by VEGF and PGF, primarily the soluble form of VEGFR-1, which negatively regulates angiogenesis. Under pathological conditions VEGF primarily binds to VEGFR-2 while PGF primarily binds to membrane-bound VEGFR-1, and both VEGF receptors promote angiogenesis after a pathological insult.

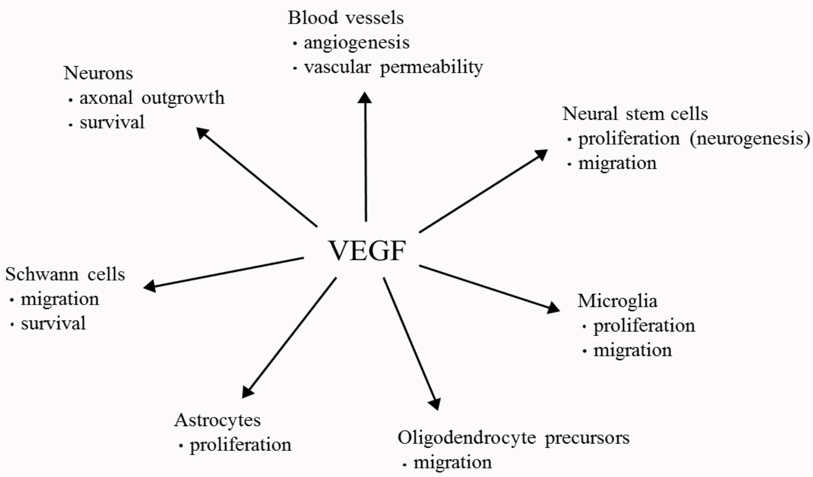

Figure 3: VEGF pleiotropism: Influence of VEGF on vasculature, neurons, neural stem cells, and glial cells (astrocytes, Schwann cells, microglia, and oligodendrocyte precursors).

epididymis, fetus, leydig cells, monocytes, ovaries, pancreas, placenta, prostate, seminiferous tubule, Sertoli cells, testis, and urothelium (HPRD: 01297, ID: 10529). VEGFR-2 is expressed in the bone marrow, heart, hematopoietic stem cells, mammary gland, neurons, placenta, testis, and urothelium (HPRD: 01867, ID: 03076). Neurons more widely express VEGFR-2 while VEGFR-1 is more abundant on glial cells [19]. As VEGF-A_{165} is the most abundant and most biologically active (pro-angiogenic) isoform of VEGF molecules, the remainder of this review will primarily focus on VEGF-A_{165} ($VEGF_{165}$) and its therapeutic application for spinal cord injury repair.

Synergistic Activation of VEGF Receptors

In 2001, Carmeliet and colleagues observed synergistic activation of the VEGFR-1 receptor by VEGF and PGF to promote angiogenesis [51] (Figure. 2). During embryogenesis, VEGFR-1 is primarily a soluble receptor, which inhibits angiogenesis by binding VEGF and thus preventing VEGF from binding to the cell-surface VEGFR-2, which promotes angiogenesis [52]. PGF binds to both the membrane-bound VEGFR-1 and the soluble inhibitory form of VEGFR-1. Thus, during embryogenesis, PGF can bind to the soluble form of VEGFR-1 and allow VEGF to bind to the membrane-bound VEGFR-2 to promote angiogenesis. In contrast, under pathological conditions VEGFR-1 is primarily membrane-bound on endothelial cells, and PGF is upregulated. Thus, PGF can activate VEGFR-1 while VEGF binds VEGFR-2, both promoting angiogenesis. Carmeliet et al. described a synergistic effect on the promotion of angiogenesis when PGF activated the membrane-bound VEGFR-1 while VEGF activated the membrane-bound VEGFR-2 [51]. While PGF and

Table 2: VEGF ligand and receptor tissue and tumor expression patterns.

Isoform/Receptor	Tissue expression	Tumor expression
VEGF-A	lungs, heart, adrenal, kidneys, liver, spleen, gastric mucosa	breast, colorectal, non-small cell lung, prostate
VEGF-B	heart,brain,kidney, testes, spleen, lung, liver	breast carcinoma, non-Hodgkins lymphoma, fibrosarcoma, benign thymoma, melanoma
VEGF-C	heart, skeletal muscle, ovaries, placenta, small intestines, lung, kidney, spleen, pancreas, prostate, testes	breast, colon, cervix, stomach, lung, prostate
VEGF-D	heart, colon, kidney, lung, liver, small intestines, ovaries, pancreas, prostate, skeletal muscles, testis, spleen	cervical intraepithelial neoplasia, colorectal, breast, glioblastoma, gastric mucosa, melanoma, thyroid carcinoma, lymph node metastasis with lung, ovarian, colorectal tumors
VEGF-E	Non-human/non-applicable	non-human protein/non-applicable
PGF	dentine matrix, endometrium, placenta, natural killer cells, serum, trophoblasts, vascular endothelial cells, heart, skin, retina, skeletal muscle	non-smell cell lung cancer, breast, gastric, prostate
VEGFR-1	endothelial cells, embryonic cells derived from endothelium (early yolk sac mesenchyme); soluble and membrane-bound forms	medullablastoma, pancreas, bladder, melanoma, Kaposi sarcoma, ovarian, prostate, malignant mesothelioma, esophageal carcinoma
VEGFR-2	endothelial cells, mononusclear cells, soluble and membrane-bound forms	medullablastoma, pancreas, bladder, melanoma, Kaposi sarcoma, ovarian, small cell lung cancer, malignant mesothelioma, myeloid leukemia,esophageal carcinoma
VEGFR-3	corneal dentric cells, retina, microvascular endothelial cells in early development, lymphatic endothelium in later development and postnatally	small cell lung cancer
NP-1	umbilical vein endothelial cells, heart, sensory, and sympathetic neurons , adipocytes, osteoblasts, bone marrow fibroblasts, microvascular endothelial cells, soluble forms - hepatocytes, renal proximal and distal tubules	prostate, colon adenocarcinoma, breast astrocytoma, esophagus, stomach, pancreas, gall bladder, giloma, neuroblastoma, melanoma, non-small cell lung cancer, small cell lung cancer
NP-2	human umbilical vein endothelial cells, sympathetic neurons, many hindbrain nuclei, inner ear, dorsal aorta, limb buds, lung, back and tongue muscles, submandibular gland, kidney, intestinal epithelium, whisker follicles	glioblastoma, neuroblastoma, melanoma, bladder, prostate, pancreas, non-small cell lung cancer, small cell lung cancer

VEGF both activate VEGFR-1, PGF results in the phosphorylation of tyrosine residue 1309 while $VEGF_{165}$ promotes the phosphorylation of tyrosine 1213 [53].

Changes in VEGF Levels and VEGF Receptor Expression after SCI

Bartholdi et al. and Herrera et al. observed reduced VEGF levels at injury epicenter at 1 day post SCI with diminished VEGF levels as far as 1 month post SCI [54,55]. Additionally, Ritz et al. reported reduced levels of VEGF, Angiopoietin-1 (Ang-1), PDGF-BB, and PGF, and increased expression of the angiogenic factor hepatocyte growth factor (HGF) [56]. VEGF receptors Flt-1 and Flk-1 have been shown to be constitutively expressed by vascular endothelial cells, neurons, and some astrocytes in the spinal cord [57]. Following SCI, VEGFR1 (Flt-1), VEGFR2 (Flk-1) and neuropilin-1 receptors have been shown to be upregulated in reactive astrocytes and microglia/macrophages following contusive SCI [57;58]. This receptor expression peaked between 7 and 14 days following injury and remained relatively high even at 14 days and beyond [57]. Taken together, this suggests that VEGF and its two tyrosine kinase receptors play a role in inflammation and the astrocytic response following contusive SCI. However, Skold et al. *in vitro* study suggests that upregulation of VEGF, its receptors and co-receptors in astrocytes may occur in the absence of inflammatory cells, with prostaglandins being upstream of VEGF [58].

Studies Employing VEGF for Repair of SCI

VEGF has become a therapeutic target for SCI repair primarily over the past two decades. Fassbender et al. nicely reviewed the literature on microvascular dysfunction following SCI, and detailed the importance of putative therapeutic approaches targeting the microvasculature [59]. VEGF routes of administration vary including exogenously applied (intrathecal and intraspinal injections, osmotic mini pumps; [18,55,60]); engineered transcription factor activation of endogenous VEGF expression [61]; overexpression via cells [62], viral vectors [17,63], or in response to other neurotrophic factor administration [21,64], or as a result of shockwave therapy [65], amongst others. VEGF has been shown to be neuroprotective [17]; promote angiogenesis [17,63,66], oligodendrogenesis and improved myelin integrity [67]; reduce tissue lesion volume [18] and increase white matter [17] and gray matter [63] sparing; promote neuritogenesis into the lesion [66]; decrease glial scar [18]; and improve locomotion [17, 61,62,65].

However, the time window of treatment onset, number of doses and duration of treatment, and VEGF dosage are crucial factors in employing this trophic factor following SCI, as some studies have reported exacerbation of lesion and decreased motor performance compared to controls [60], aberrant excessive sprouting of axons [68] and increased mechanical allodynia [68,67]. Drs. Benton and Whittemore administered a supraphysiological dosage of VEGF (0.5 μg/μL) at 3 days post injury; considering the peak of the inflammatory phase and the very high VEGF dosage, it is reasonable to have observed exacerbation of lesion, likely due to excessive vascular permeability and extravasation of inflammatory mediators. It is unknown whether the increased mechanical allodynia in these studies is a result specifically of $VEGF_{165}$ or perhaps $VEGF_{188,}$ as suggested by Nesic et al. [68]. However, it is noteworthy that a subset of saline injected SCI control animals also developed mechanical allodynia [67] similar to other studies [63]. Thus, VEGF may just be one of the key players involved in mechanical hypersensitivity after SCI. Interestingly, van Neerven et al [69]. had a similar route of intrathecal VEGF administration as Sundberg et al. [67]; however, van Neervan gave daily injections for the first week post-SCI while Sundberg's group gave only one injection immediately following injury. Sundberg and colleagues observed exacerbated forepaw mechanical allodynia [68]; yet van Neervan and colleagues observed a decrease in mechanical allodynia of the hindpaw [69]. Additionally, Figley et al. reported significantly decreased mechanical allodynia in VEGF treated rats compared to saline or viral vector vehicle controls [63]. Observed differences across these studies are likely due to the duration of VEGF administration and dosages.

In a study of cerebral ischemia, Manoonkitiwongsa et al. reported neuroprotection with low (2μg) and medium (8μg) doses of $VEGF_{165}$, subthreshold to promote angiogenesis [70]. However, higher (60μg) doses of $VEGF_{165}$ resulted in angiogenesis without neuroprotection in ischemic brains and neuronal injury in $VEGF_{165}$ treated non-ischemic (uninjured/normal) brains. This study further demonstrates the crucial aspect of VEGF dosage, in addition to timing, particularly for studies targeting angiogenesis and neuroprotection concomitantly. Shinozaki et al. [71] investigated the contributions of VEGFR-1 and VEGFR-2 activation on neuroprotection following SCI, through neutralizing antibodies, and determined VEGFR-1 plays a major role in vascular permeability, while VEGFR-2 promotes neuron survival.

Studies Using VEGF in Combinational Therapies for SCI Repair

Similar to other neuroprotective and neural regeneration individual therapies, VEGF alone might be insufficient to produce significant axon regeneration/sparing, functional synapse formation, and improved functional recovery following SCI. Thus, further investigation of VEGF is necessary, and more studies are employing VEGF as part of combinational treatments. In 2012, Lutton et al. showed the promising combination of VEGF and PDGF after SCI reduced lesion cavity, glial scar density, and the inflammatory response (macrophage/microglia) [72]. This combination of trophic factors (VEGF and PDGF) was also shown to promote improved functional recovery following SCI [73]. Gong et al. [74] observed neuroprotection of spinal cord neurons through VEGF, specifically VEGFR-2 (Flk-1), after application of the endothelin-A/B dual receptor antagonist (Bosentan). In a 2011 combinational study [75], poly (lactide-co-glycolide) (PLG) bridges loaded with VEGF and FGF-2 (fibroblast growth factor 2) promoted neurite growth and angiogenesis within the lesion site, and prevented the formation of cystic cavity. Additional trophic factors might be necessary in order to promote significant axonal re-growth and functional recovery.

VEGF Neuroprotection for Other Neurodegenerative Disease Models

Dysfunctional vasculature or aberrant VEGF levels negatively influence a number of neurodegenerative diseases including Alzheimer's disease, Amyotrophic Lateral Sclerosis (ALS; Lou Gehrig's disease), Huntington's disease, Parkinson's disease, and stroke [16]. In a mouse model of epilepsy, VEGF administration preserved learning and memory function (Morris water maze) and reduced anxiety-like behaviors typically observed in status-epilepticus rodents [76]. Reduced VEGF levels can lead to rodent ALS-like phenotypes: decline of motor function and decreased grooming behavior in a SOD1 (Superoxide dismustase 1) mouse model of ALS [14], and with deletion of the HRE (hormone response element) in the VEGF promoter [77]. In similar studies of human patients, Carmeliet & Ruiz de Almodovar [14] and Lambrechts et al. [53] determined that human ALS patients had lower VEGF levels compared to healthy population-based controls, with the lowest VEGF serum levels correlating with the greatest ALS susceptibility [53]. Additionally, VEGF was shown to be neuroprotective in rodent models of Parkinson's disease [21], ALS [22], Huntington's disease [23], and cerebral ischemia [70,78]. Moreover, VEGF-A165 competes with Semaphorin 3A for signaling through the neuropilin-1 receptor, for promoting axonal outgrowth and chemoattraction versus axonal guidance by chemo repulsion, respectively [15]. Thus, inhibition of Semaphorin 3A is another putative target for SCI therapies.

Potential Signaling Mechanisms of VEGF for Neuroprotection and Tissue Repair Following SCI

VEGF also influences many cell types, including neurons [79,80,81], Schwann cells [82], astrocytes [83,84], microglia, neuronal stem cells [85,86], and oligodendrocyte precursors, to promote angiogenesis, neurogenesis [87], dendritogenesis, synaptic plasticity, axon growth and guidance, cell survival [78], proliferation [88], migration, differentiation, neuromuscular junction innervation, and neuroendothelial junction maintenance (Figure 3). Jin et al. [89] detailed the neuroprotective effects of hippocampal neurons by VEGF activation of VEGFR-2, and downstream signaling of PI3K, with reduced caspase-3. Hao and Rockwell [90] showed the neuroprotection of hippocampal neurons via signaling through VEGF activation of VEGFR-2, with downstream signaling through the PI3K/Akt and MEK/ERK pathways. This study also suggests that VEGFR-1 and NP-1 likely serve as backup signaling pathways for neuroprotection with blockade of VEGFR-2. The pleiotropic mechanisms of VEGF are summarized in Figure 3, as well as Storkebaum et al. [16], Nowacka and Obuchowicz et al. [91], and Carmeliet and Ruiz de Almodovar [14].

Conclusion

The pleiotropic mechanism of VEGF (migration, proliferation, cell survival) upon many different cell types (endothelial cells, neurons, astrocytes, Schwann cells, neural stem cells, microglia, and oligodendrocytes) makes this trophic factor a prime putative target for many neurodegenerative diseases and condition of the nervous system (Figure 3). These include Parkinson's, Huntington's, and Alzheimer's disease, Amyotrophic Lateral Sclerosis (ALS), stroke/ischemia, diabetic neuropathy, as well as traumatic brain and spinal cord injuries. In addition to its canonical role in the cardiovascular system, VEGF has also been shown to promote neuroprotection, axonal guidance, Schwann cell survival and migration, and proliferation of astrocytes, microglia, and neural stem cells. VEGF has high therapeutic potential particularly for spinal cord injury, for promoting spinal cord revascularization, neuroprotection, cell proliferation, and tissue regeneration, ultimately for improved functional recovery.

Since the discovery of VEGF in early 1970's, by Dr. Judah Folkman [11] and its official naming by Drs. Ferrara and Henzel [28], it has been determined that this vascular trophic factor has much broader implications than its canonical role in development of the vascular system. VEGF's pleiotropic mechanisms include: angiogenesis [11,12], axonal guidance [13,14,15], neuroprotection [16,17,18,19], Schwann cell survival and migration, and proliferation of astrocytes, microglia, and neural stem cells [16]. Moreover, deletions within the VEGF promoter region cause a neurodegenerative phenotype in mice, similar to Amyotrophic Lateral Sclerosis (ALS), showing VEGF is important for maintenance of motor function [77]. Additionally, Lambrechts et al. [92] showed motoneuron protection by VEGF administration in an ALS mouse model. This study also showed that VEGF serum levels in European patients correlated with ALS susceptibility, with lower circulating VEGF levels correlating with higher risk of sporadic ALS. VEGF delivered via a retroviral vector delayed disease onset, promoted neuroprotection, and prolonged survival of animals with an ALS phenotype [22]. Similarly, VEGF delivered intracerebroventricularly prolonged the survival period, delayed the disease onset, and spared motor neurons in an ALS model [93].

After spinal cord injury, an angiogenic response occurs that peaks approximately 7-14 days post-injury, and regresses coincident with the onset of cystic cavitation, in both rats and higher primates [9,10,59]. Intact vasculature is crucial for delivering oxygen and nutrients to the tissues and for removing toxic wastes. In studies of SCI, VEGF has been shown to: 1) promote angiogenesis [17,63,66], 2) decrease the glial scar [18], 3) increase white matter sparing [17], 4) increase gray matter sparing [63], 5) promote neuroprotection [17], 6) promote neuritogenesis into the lesion [66], 7) promote oligodendrogenesis and improved myelin integrity [67], 8) reduce tissue lesion volume [18], and 9) promote improved locomotion [17,69,61,65]. Therefore, VEGF appears to be a promising target for repair of the injured nervous system, due to trauma and degenerative diseases.

However, the main factors for consideration in applying this trophic factor in models of SCI are time point of administration and VEGF concentration, as some studies have observed exacerbation of SCI lesion [60], likely due to early time point application after SCI insult and supraphysiological doses of VEGF. It is also important to consider that VEGF administered alone might be insufficient to promote neuroprotection, axon regeneration/sparing, functional synapse formation, and improved functional recovery following SCI. Current literature suggests that VEGF in combination with other therapeutic approaches for SCI appears to hold the greatest potential for promoting angiogenesis, neuroprotection, axonal regeneration, and functional recovery [73-75].

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