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Review Article

Anti-VEGF treatment improves neurological function in tumors of the nervous system

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ABSTRACT

Research of various diseases of the nervous system has shown that VEGF has direct neuroprotective effects in the central and peripheral nervous systems, and indirect effects on improving neuronal vessel perfusion which leads to nerve protection. In the tumors of the nervous system, VEGF plays a critical role in tumor angiogenesis and tumor progression. The effect of anti-VEGF treatment on nerve protection and function has been recently reported - by normalizing the tumor vasculature, anti-VEGF treatment is able to relieve nerve edema and deliver oxygen more efficiently into the nerve, thus reducing nerve damage and improving nerve function. This review aims to summarize the divergent roles of VEGF in diseases of the nervous system and the recent findings of anti-VEGF therapy in nerve damage/regeneration and function in tumors, specifically, in Neurofibromatosis type 2 associated schwannomas.

1. Introduction

Vascular endothelial growth factor (VEGF) is a multifunctional cytokine, originally discovered as a tumor-secreted protein that promotes vascular permeability (Dvorak et al., 1999). VEGF promotes angiogenesis by inducing migration and proliferation of endothelial cells (Carmeliet and Jain, 2000; Ferrara, 1999). It is expressed in virtually all tumor types, and is correlated with angiogenesis, tumor growth, invasion and metastasis (Carmeliet and Jain, 2000). The critical role of VEGF in angiogenesis and tumor growth has been proven mechanistically by the targeted deletion of the *Vegfa* gene (Grunstein et al., 1999; Tsuzuki et al., 2000), by blocking antibodies (Yuan et al., 1996) and by introduction of antisense *VEGF* constructs into neoplastic cells (Oku et al., 1998; Xu et al., 2002).

A number of studies have examined the effects of VEGF in the nervous system (Lambrechts and Carmeliet, 2006; van Bruggen et al., 1999; Zhang et al., 2000). During embryonic development, VEGF is expressed in the ventricular zone, and the VEGF receptors are expressed in endothelial cells of the perineural capillary plexus and capillary sprouts, infiltrating into the neuroectoderm (Breier et al., 1995). In the adult brain, VEGF, as well as its receptors, VEGF-R1, -R2 and neuropilin-1 (NRP-1), are expressed in a region-specific manner in glial cells (Acker et al., 2001; Barouk et al., 2011; Bengoetxea et al., 2008; Licht et al., 2010; Licht et al., 2011), neurons (Li et al., 2009) and Purkinje cells (Maharaj et al., 2006; Ruiz de Almodovar et al., 2010). A great number of reports have shown that VEGF family members exert versatile effects in the nervous system - stimulating neural cell proliferation, migration, differentiation and survival during development and in the adult (Calvo et al., 2011; Falk et al., 2011; Jin et al., 2002; Le Bras et al., 2006; Licht et al., 2010; Licht et al., 2011; Louissaint et al., 2002; Palmer et al., 2000; Ruiz de Almodovar et al., 2010; Schanzer et al., 2004; Sondell et al., 1999; Sun et al., 2006; Wittko et al., 2009). In recent years, numerous studies have examined the neurotrophic and neuroprotective roles of VEGF in disease of the nervous system, such as neurodegenerative disorders (Parkinson's disease and amyotrophic lateral sclerosis (ALS)), ischemic brain injury (e.g., stroke), peripheral nerve injury, and retinal diseases (Carmeliet, 2003; Ferrara et al., 2003; Raab and Plate, 2007; Ruiz de Almodovar et al., 2009) (Table 1).

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Table 1
VEGF signaling in diseases of the nervous system.

Disease	Model	Nerve/neuron	Method to assess VEGF function	Findings	Mechanisms	References
			Neurodegenerative disorders			
ALS	Mouse and rat neuron culture	Motor neuron of spinal cord	Genetic mutation: $Vegf^{\partial/\partial}$	VEGF enhances survival of motor neurons	VEGF protects ischemic motor neuron from death through the VEGF-R2	(Van Den Bosch et al., 2004)
ALS	• Human blood sample • Mouse spinal cord ischemia model	Motor neuron of spinal cord	Genetic mutation: $Vegf^{\partial/\partial}$	Reduced VEGF predispose to motor neuron loss in human and mice Intraperitoneal administration of VEGF protects against loss of motor neurons after stress	• *Vegfa* gene mutation leads to severe motor neuron degeneration • *Vegfa* protected mice against ischemic motor neuron death	(Lambrechts et al., 2003)
ALS	Mouse model	Motor neuron of spinal cord	Genetic mutation: $Vegf^{\partial/\partial}$	Low VEGF level leads to progressive motor neuron degeneration and muscle atrophy	Reduced VEGF expression and abnormal vasculature in $Vegf^{\partial/\partial}$ transgenic mice lead to reduced neural vascular perfusion and insufficient $Vegf_{165}$-dependent neuroprotection	(Oosthuyse et al., 2001)
AD	Rat cerebral ischemia model	Cerebral cortex	Expression analysis	Increased VEGF expression in cerebral cortex of ischemic and chronic hypoxic cerebral	Enhanced VEGF immunoreactivity in clusters of reactive astrocytes and cerebral vessels in both mouse and human brain tissue	(Kalaria et al., 1998)
Ischemic brain injury						
Stroke	Rat focal cerebral embolic ischemia model	CNS	Motor and sensory function test	Recombinant VEGF treatment enhances angiogenesis and improves neurological recovery	VEGF treatment induces capillary formation and increases cerebral microvascular plasma perfusion in the penumbra of the cortex	(Lennmyr et al., 1998; Zhang et al., 2000)
Stroke	Mouse neuron cell line	Hippocampal neuron	Hypoxia and glucose deprivation rescue in vitro study	VEGF reduces cell death of hippocampal neurons	VEGF mediates neuroprotective effects via VEGF-R2, PI3-K/Akt signaling	(Jin et al., 2000)
Stroke	Mouse brain ischemia model	CNS	Expression analysis	Antagonist of VEGF reduces brain edema and injury	VEGF antagonist reduces vessel edema and permeability	(van Bruggen et al., 1999)
Cerebral ischemia	Mouse neuron culture	Cortical neuron	Hypoxia rescue in vitro study	VEGF acts as an neuroprotective factor in cerebral ischemia	VEGF inhibits the activation of caspase-3 under hypoxic condition	(Jin et al., 2001)
			Periphery nerve injury			
Injury	• Mouse neuron culture • Mouse peripheral nerve injury model	Corneal nerve	• Genetic knock-out: $Vegf\text{-}b^{-/-}$ • In vitro neuron growth	VEGF-B stimulates nerve regeneration and recovery of tissue sensation	Neurotrophic effect of VEGF-B via PI3K and Notch signaling	(Guaiquil et al., 2014)
Injury	• Mouse neuron culture • Mouse peripheral nerve injury model	Corneal nerve	Sensory functional test	VEGF mediates growth of trigeminal neurons	Neurotrophic effect of VEGF via activating VEGF-R1, VEGF-R2, and NRP-1	(Pan et al., 2013)
Injury	Mouse/rat sciatic nerve transection model	Sciatic nerve	• Behavioral test • Functional test	VEGF enhances the growth of regenerating nerve fibers	Combination of enhanced angiogenic, neurotrophic and neuroprotective effects	(Mohammadi et al., 2013; Pereira Lopes et al., 2011)
Injury	• Mouse corneal nerve damage model • Mouse trigeminal ganglia explant culture • Mouse neuron culture	Corneal nerve	Expression analysis	Anti-VEGF treatment inhibits the repair of corneal nerves	Anti-VEGF reduces neuron growth and regeneration	(Yu et al., 2008)
Injury	Rat MPG culture	Pelvic nerve	Neurite outgrowth	VEGF promotes MPG fiber outgrowth	VEGF induces NOS and TH in neurons	(Lin et al., 2003)
Injury	Mouse cavernous nerve neurotomy model	Cavernous nerve	• Neurite outgrowth • Erectile function evaluation	Intracavernous injection of VEGF facilitates the recovery of erectile function	VEGF facilitates axon growth via upregulating nNOS expression	(Lin and Lue, 2004)
Ischemic injury	Mouse peripheral nerve injury model	Sternomastoid muscle nerve	Motor end-plate innervation evaluation	VEGF supplementation results in angiogenic and neurogenic responses	VEGF induces expression of NGF and GDNF	(Shvartsman et al., 2014)
Ischemia injury	Rabbit hind limb ischemia model	Peroneal nerve	• Nerve conduction • Schwann cell migration	Intramuscular VEGF gene transfer recovers nerve function	VEGF enhances vessel perfusion	(Schratzberger et al., 2000)
Peripheral nerve injury	Mouse SCG and DRG explant culture models	SCG, DRG	Neurite outgrowth	VEGF increases survival and proliferation of neurons, Schwann cells		(Sondell et al., 1999)

(*continued on next page*)

Table 1 (*continued*)

Disease	Model	Nerve/neuron	Method to assess VEGF function	Findings	Mechanisms	References
					VEGF improves survival and increases proliferation of Schwann cells through VEGF-R2 and the MAPK pathway	
				Retinal disease		
Glaucoma	• Rat retinal ganglion cell culture model • Rat experimental hypertensive glaucoma model	Retinal ganglion cells	Neurite outgrowth	VEGF-A promotes retinal ganglion cells survival	Neuroprotective effect of VEGF via VEGF-R2 and PI3K/Akt pathway	(Foxton et al., 2013)
Inflammation	Mouse corneal abrasion model	Corneal nerve	Anti-VEGF treatment	Anti-VEGF administered systemically and locally retards nerve regeneration	Anti-VEGF reduces inflammatory response involving neutrophils and platelets	(Li et al., 2011)
Retinal ischemia	Mouse ischemia-reperfusion injury	Optic nerve	• Retinal thickness evaluation • Anti-VEGF treatment	VEGF directly recues retinal cell apoptosis VEGF increases blood flow	Direct neuroprotective effect of VEGF via VEGF-R2 VEGF increases blood flow via iNOS	(Nishijima et al., 2007)
Retinal ischemia	Rat retinal explant model	Retinal explant	Anti-VEGFR treatment	VEGF induces neurites outgrowth of retinal explant	VEGF stimulates axonal outgrowth via VEGF-R2	(Bocker-Meffert et al., 2002)
				Tumors of the central nervous system		
GBM	• Mouse cranial window model • Phase II clinical trial	Brain	Anti-VEGFR treatment	Anti-VEGF treatment improves survival of the mice by controlling edema	Anti-VEGF treatment normalizes tumor blood vessels High dose of bevacizumab has direct anti-tumor effect	(Kamoun et al., 2009) (von Baumgarten et al., 2011)
GBM	Orthotopic xenograft model	Brain	Anti-VEGFR treatment	Combined anti-VEGF and TMZ treatment inhibits tumor growth	Combined anti-VEGF treatment can enhance TMZ-induced apoptosis through specific down-regulation of NRP-1	(Lee et al., 2016; Son et al., 2006)
GBM	Mouse orthotopic xenograft model	Brain	Anti-VEGFR treatment	Radiation is most effective when administered during the "normalization window" induced by anti-VEGF treatment	VEGFR2 blockade transiently normalizes brain tumor vessels via upregulation of Ang1 and MMP activation	(McGee et al., 2010; Verhoeff et al., 2009; Winkler et al., 2004)
GBM	Phase II clinical trials (NCT00345163, NCT00393094, NCT00613028)	rGBM patients	Clinical trail	Anti-VEGF treatment leads to significant radiographic response rates and improved PFS	Anti-VEGF treatment induces normalization of brain tumor vasculature	(Friedman et al., 2009; Kreisl et al., 2009)
GBM	Phase III clinical trials (NCT00884741, NCT00943826)	nGBM patients	Clinical trail	Anti-VEGF treatment combined with radiotherapy and TMZ improves PFS No improvement in median OS	Anti-VEGF treatment induces normalization of brain tumor vasculature	(Chinot et al., 2014; Gilbert et al., 2014; Vredenburgh et al., 2007)
Schwannomas						
NF2 Schwannoma	Mouse orthotopic xenograft model	• Sciatic nerve • Intracranial model	Anti-VEGF treatment	Anti-VEGF treatment enhances tumor inhibition, decreases dose of radiation, and improves neurological function	Anti-VEGF treatment normalizes tumor vasculature, reduces tumor and nerve edema, increases nerve regeneration, decreases muscle atrophy and improves neurological function	(Gao et al., 2015)

Abbreviations: CNS, central nervous system; BBB, brain blood barrier; VEGF, vascular endothelial growth factor; VEGF-R1, VEGF receptor 1; VEGF-R2, VEGF receptor 2; VEGF-A, vascular endothelial growth factor A; VEGF-B, vascular endothelial growth factor B; NRP-1, neuropilin 1; PI3K, phosphatidylinositol 3-kinase; MPG, major pelvic ganglia; NOS, nitric oxide synthase; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; NGF, nerve growth factor; GDNF, glial derived neurotrophic factor; SCG, superior cervical ganglia; DRG, dorsal root ganglia; MAPK, mitogen activated protein kinase; AD, Alzheimer's disease; TH, tyrosine hydroxylase; GBM, glioblastoma; TMZ, temozolomide; Ang1, angiopoietin-1; MMP, matrix metalloproteinase; PFS, progression-free survival; OS, overall survival, rGBM, recurrent GBM; nGBM, newly diagnosed GBM.

However, its role and potential as a therapeutic target in tumors of the nervous system remain unknown.

2. Role of VEGF in the nervous system

In various disease models, the effect of VEGF on nerves is two fold: 1) a direct neuroprotective effect on neurons (Jin et al., 2001; Jin et al., 2000; Lambrechts and Carmeliet, 2006); and 2) an indirect angiogenic effect that provides an "angiogenic niche", which improves neural perfusion and favors neuronal progenitor proliferation and differentiation *in vivo* (Hoke, 2006; Lambrechts and Carmeliet, 2006; Palmer et al., 2000; Webber and Zochodne, 2010).

The level of VEGF has been demonstrated to be a factor in neurodegenerative disorders. ALS is a progressive, adult-onset neurodegenerative disease characterized by degeneration and loss of the large motor neurons in the cerebral cortex, brainstem and spinal cord, leading to muscle atrophy, paralysis and death (Al-Chalabi et al., 2017). The clinical symptoms and neuropathological signs of ALS have been successfully reproduced in a genetic mouse model in which the hypoxia response element (HRE) of the *Vegfa* promoter is mutated. The abolishment of hypoxic regulation of VEGF leads to significantly reduced spinal VEGF levels, which results in decreased neural perfusion, with spinal cord ischemia, and ultimately leads to both motor neuron degeneration and progressive paralysis (Lambrechts et al., 2003). This study exhibited that the vascular function of VEGF plays an important role in the nervous system homeostasis. In other late-onset neurodegenerative diseases including Alzheimer's dementia and Parkinson's disease, substantial evidence has shown that decreased cerebral perfusion becomes significant during aging. The reduced blood supply and impeded delivery of oxygen and metabolism of glucose leads to a chronic mismatch between blood flow and neural energy consumption, which may destabilize neurons and induce neurodegeneration (de la Torre, 2000; Farkas et al., 2000) (Table 1).

In ischemic brain injury (stroke), expression of VEGF is highly upregulated after onset of ischemia (Plate et al., 1999). In a rat middle cerebral artery occlusion (MCAO) model, administration of recombinant VEGF 24 h after ischemia (via intracerebroventricular route) demonstrates enhanced cerebral angiogenesis and microvascular perfusion, and significantly improves neurological recovery and reduces damaged brain volume (Guaiquil et al., 2014; Sun et al., 2003; Zhang et al., 2000). In addition to the vascular effects, VEGFR-1 and R2 are also upregulated in neurons and glial cells, and NRP-1 is also upregulated in neurons and astrocytes surrounding the infarct. Furthermore, VEGFR-2 has been shown to mediate the neuroprotective effects of VEGF via PI3-K/Akt signaling (Table 1). These studies suggest that VEGF may be directly involved in neuroprotection during stroke recovery (Jin et al., 2000).

In peripheral nerve injury, VEGF has been identified as a signaling factor that facilitates the crosstalk between the neural and vascular systems. In a sciatic nerve transection model, local application of VEGF accelerates functional recovery (Mohammadi et al., 2013); furthermore, it has been shown that administration of VEGF supports and enhances the growth of regenerating nerve fibers, through a combination of angiogenic, neurotrophic and neuroprotective effects (Pereira Lopes et al., 2011). Interestingly, VEGF accelerates nerve growth only in regenerating nerves, which results in more rapid return of sensation and neurotrophic effects. This effect has been found to require the activation of multiple VEGF receptors, VEGFR1, VEGFR2, and NRP-1 (Pan et al., 2013) (Table 1).

The cornea is among one of the most densely innervated tissues of the human body. In diseases of the eye, recombinant VEGF directly promotes the growth of nerve processes from trigeminal ganglia explants *in vitro*, whereas anti-VEGF antibody (bevacizumab) reduces cultured axon growth (Yu et al., 2008). In the neurofluorescent thy1-YFP mouse, it has been found that the trigeminal ganglion expresses VEGF and its receptors VEGF-R1, VEGF-R2, NRP-1, and NRP-2, confirming that the trigeminal neurons have the receptors to respond to VEGF *in vivo*. Indeed, in a corneal epithelium nerve damage model, bevacizumab treatment significantly inhibits the repair of the nerves. Consistent with these findings, studies of ocular vascular diseases using optical nerve ischemia-reperfusion injury model have found that VEGF has a direct survival effect on neuronal cells of the retina, independent of blood flow, and that VEGFR2 activation is sufficient to trigger retinal neuroprotection (Bocker-Meffert et al., 2002; Nishijima et al., 2007). In models of experimental glaucoma, VEGF, via the PI3K/Akt pathway, also acts directly on retinal ganglion cells (RGCs) to promote survival (Foxton et al., 2013) (Table 1). In eye disease, the disruption of the corneal nerves has been shown to significantly impair corneal healing; therefore, these studies suggest a cautious use of the anti-VEGF treatment in disease of the eye.

Taken together, these findings suggest that VEGF may ameliorate the adverse clinical outcomes of stroke, peripheral nerve injury and neurodegenerative disorders. However, approaches utilizing VEGF administration have had limited success, likely due to the rapid clearance of VEGF protein delivered in a solution form. Infusion of VEGF in clinical trials resulted in elevated VEGF plasma levels during the infusion, but this was followed by a rapid clearance of VEGF once infusions were discontinued (Eppler et al., 2002). Studies aimed to improve drug delivery have shown that poly(lactic-*co*-glycolic) acid (PLGA) microparticles as carriers for VEGF helped to preserve the VEGF bioactivity in a rat myocardial infarction model (Simon-Yarza et al., 2013). However, more preclinical studies are needed to evaluate the possible therapeutic effect on improving neurological function and the side effects of chronical administration of VEGF in these diseases.

3. Effect of anti-VEGF treatment in tumors of the central nervous system

Compared to the above-mentioned diseases of the nervous system, the role of VEGF on neurological functions in patients with tumors of the nervous system remains to be elucidated. Multiple preclinical and clinical studies have reported potential roles of VEGF on the progression of malignant brain tumors (Lu-Emerson et al., 2015). In glioblastoma (GBM) preclinical models, anti-VEGF treatment enhances the efficacy of chemotherapy and radiation therapy via normalization of the tumor vasculature, thus improving the delivery of chemotherapeutic drugs and oxygen (Kamoun et al., 2009; McGee et al., 2010). Initial phase II studies in recurrent GBM (rGBM) patients demonstrated promising results with significant radiographic response rates and improved progression-free survival (PFS) achieved with bevacizumab therapy (Ferrara et al., 2004; Friedman et al., 2009; Kreisl et al., 2009; Vredenburgh et al., 2007). On the basis of these results, the US Food and Drug Administration granted approval for the use of bevacizumab in rGBM in 2009. However, two subsequent randomized, placebo-controlled phase III trials of bevacizumab with chemoradiotherapy in patients with newly diagnosed GBM (nGBM) (NCT00884741 and NCT00943826) failed to demonstrate an improvement in OS (Chinot et al., 2014; Gilbert et al., 2014) (Table 1). Interestingly, these two studies reported conflicting results regarding the quality of life and cognitive function in the setting of bevacizumab treatment. Chinot et al. reported maintenance of baseline quality of life and performance status with bevacizumab treatment, and lower glucocorticoid requirement (NCT00943826) (Chinot et al., 2014), while Gilbert et al. reported a worse quality of life, and a decline in neurocognitive function in the bevacizumab group (NCT00884741) (Gilbert et al., 2014). Differences between these two studies may potentially come from variations in recording neurocognitive function, as Trial #NCT00943826 evaluated patient-reported health-related quality-of-life measures, while Trial # NCT00884741 collected measures of symptom burden and interference and the results of objective tests of neurocognitive function. The mechanisms of anti-VEGF treatment on neurological function in these patients are less well studied. Previously, it has been shown that

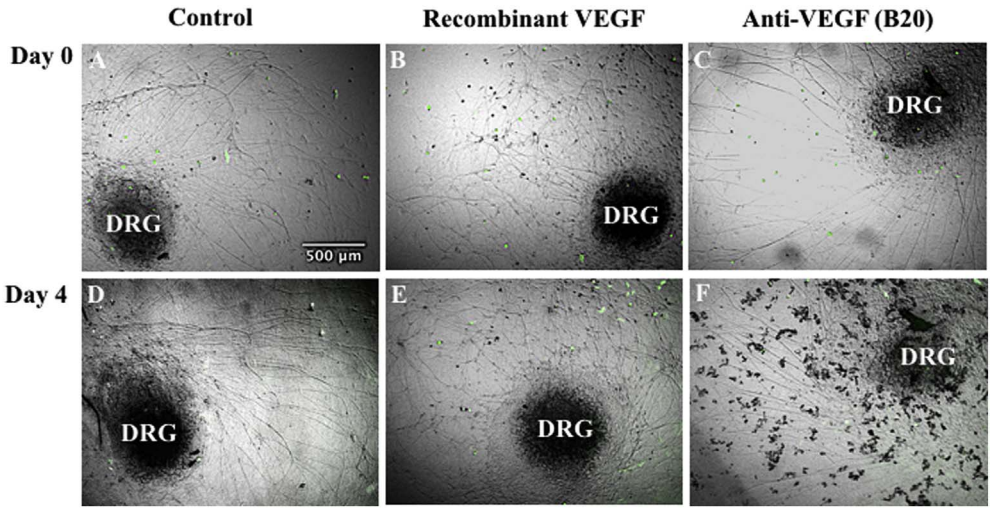

Fig. 1. Anti-VEGF treatment leads to DRG explant degradation. Postnatal 1 to 2 day-old nude mice pups were sacrificed and DRG were carefully dissected from the thoracic vertebrae down to the lumbar vertebrae under microscope. After removing the connective tissue and cut into thin pieces, DRGs were explanted onto ammoniated rat tail collagen- and poly-L-lysine-coated glass cover slips and maintain in myelination medium (Paivalainen et al., 2008). GFP-labeled schwannoma cells were seeded at the concentration of 5000 cells/well 5 days after DRG explantation. One day later, recombinant mouse VEGF (100 ng/ml, R&D Systems, Minneapolis, MN) or anti-VEGF antibody (B20, 100 μg/ml, Genentech, South San Francisco, CA) were added into the culture medium. DRGs without treatment were used as control. DRG cultures in different treatment groups were imaged at 0 day (A–C) and 4 days after treatment by phase contrast microscopy (4 × objective, Olympus IX70 microscope).

bevacizumab treatment significantly reduced brain edema, and the reduction of edema is associated with a consistently stable quality of life across all domains, sustained functional independence, and a diminished glucocorticoid requirement (Gerstner et al., 2009). These studies suggest that a careful review of the effect of bevacizumab on brain edema and neurocognitive function would be needed to understand these differences.

4. Effect of anti-VEGF treatment on nerve function in patients with NF2 vestibular schwannoma

Recent studies of anti-VEGF treatment in Neurofibromatosis type 2 (NF2) schwannomas have shed light on the role of VEGF in the progression and nerve function and regeneration in tumors of the nervous systems (Gao et al., 2015). NF2 is a dominantly inherited genetic condition with a birth prevalence of 1 in 25,000 (Evans et al., 1992). NF2 is characterized by bilateral vestibular schwannomas (VS), which are benign tumors composed of neoplastic Schwann cells that arise from the eighth cranial nerve that transmits hearing and balance information from the ears to the brain. Although these VS grow slowly, they usually lead to a significant or total hearing loss by young adulthood or middle age. The tumors can also compress the brain stem leading to headaches, difficulty swallowing, and other serious neurologic symptoms (Plotkin et al., 2014). Standard approaches for the treatment of growing VS include surgical resection and radiation therapy (RT). While these tumors can be successfully removed or destroyed with surgery and radiation treatment, paradoxically, these therapeutic approaches can also cause cranial nerve damage and associated adverse effects, including diminished hearing, swallow, and facial functions. For patients with sporadic VS who do not have NF2, RT is associated with long-term tumor control rates exceeding 95%. However, hearing preservation rates after radiation range from 50 to 80% (Ammoun and Hanemann, 2011; Kano et al., 2009; Patel et al., 2014; Plotkin et al., 2012; Subach et al., 1999; Timmer et al., 2011; Wagner et al., 2014). Post-RT outcomes for patients with NF2 are inferior to those for sporadic patients, with short-term local tumor control rates around 80–85% and hearing preservation rates less than 50% (Ammoun and Hanemann, 2011). Thus, the identification of a novel adjunct therapy to enhance radiosensitivity while minimizing toxicity-related hearing loss in VS is urgently needed.

Several previous investigations have suggested that – unlike other benign tumors – VS, are able to induce the formation of new blood vessels (di Tomaso et al., 2011; Plotkin et al., 2009), a characteristic often associated with malignant tumors. VEGF and its receptors (VEGFRs) are expressed in VS, and VEGF expression level positively correlates with schwannoma growth rate (Brieger et al., 2003; Caye-Thomasen et al., 2003; Plotkin et al., 2009). Bevacizumab has been associated with a reduction in the volume of most growing VS, and, more importantly, improved hearing in 57% patients (Blakeley et al., 2016; Plotkin et al., 2009). The fact that not all patients respond and that hearing improvement is often transient, as well as the lack of direct evidence of the effects of anti-VEGF treatment on nerve function indicate the need to better understand the mechanisms of anti-angiogenic therapy on the function of tumor-bearing nerves.

5. Mechanisms of the neuroprotective effect of anti-VEGF treatment in NF2

In a sciatic nerve model of NF2, Gao et al., have shown that anti-VEGF treatment improves neurological function. Under electron microscopy (EM), for the first time, it has been reported that anti-VEGF treatment leads to regeneration and remyelination of the nerve axons in tumor-bearing mice (Gao et al., 2015). It has been further demonstrated that the effect of anti-VEGF treatment occurred via normalization of the tumor vasculature and improvement in vessel perfusion (Gao et al., 2015). This is consistent with previous findings that have shown improved neural perfusion favors neuronal progenitor proliferation and differentiation in vivo (Hoke, 2006; Lambrechts and Carmeliet, 2006; Palmer et al., 2000; Webber and Zochodne, 2010). Furthermore, this study reports that anti-VEGF treatment can significantly alleviate perineuronal edema, which faithfully recapitulates the clinical findings in NF2 studies that patients with excess edema are most likely to benefit from bevacizumab treatment (Gao et al., 2015).

To study whether anti-VEGF treatment has a direct effect on nerves, organotypic culture models have been used (Fig. 1). Schwannoma cells are co-cultured with dorsal root ganglia (DRG) explants, and treated with control immunoglobulin, recombinant VEGF, or VEGF neutralizing antibody (B20, Genentech). The results show that anti-VEGF antibody treatment leads to significant DRG and neurite degradation. These data confirm a direct role of VEGF on neuroprotection in the tumor model. However, it raises the question: what is the dominant effect of anti-VEGF treatment in tumor models? In the NF2 schwannoma model, it has been reported that anti-VEGF treatment does not significantly decrease VEGF production (Gao et al., 2015); therefore, the direct neuroprotective effect from VEGF remains unchanged. However, anti-VEGF treatment significantly normalizes tumor vasculature and improves vessel perfusion. Therefore, the end result in this model is the dominant vascular effect that favors neuroprotection.

Anti-VEGF agents were originally developed to block tumor growth by inhibiting blood vessel formation (Carmeliet and Jain, 2011; Goel et al., 2011). However, bevacizumab has failed to improve survival benefit as a monotherapy in a number of tumors, but can confer

survival benefit in combination with chemo- or immunotherapies (Goel et al., 2011). Numerous preclinical and clinical studies have provided evidence that the success of combined therapies stems from the fact that bevacizumab "normalizes" the abnormal vasculature of tumors - the resulting vasculature is structural and functionally more normal, characterized by increased blood flow and improved delivery of oxygen (Goel et al., 2011). These studies suggest that in future clinical studies, judicious use of anti-VEGF treatment is required to achieve vessel normalization, and biomarker analysis of VEGF, its receptors and downstream signaling pathways should be considered to determine the potential outcome of nerve preservation and neurological function.

6. Summary

Research in various diseases of the nervous system has shown that VEGF has direct neuroprotective effects in the central and peripheral nervous system, as well as indirect effects in improving neuronal vessel perfusion which results in nerve protection. In tumors of the nervous system, VEGF level is significantly elevated and plays a critical role in tumor angiogenesis and progression. The effect of anti-VEGF treatment on nerve protection and function is recently reported in NF2 schwannoma model- by normalizing the vasculature, anti-VEGF treatment is able to relieve nerve edema and deliver oxygen more efficiently into the nerve, and thus reduce nerve damage and improve nerve function. A deeper understanding of the normalization process is required for anti-VEGF treatment to be more effectively exploited in restoring nerve function in the clinical setting. Because of the dual role of VEGF on nerve function, in future studies of both tumor and non-tumor associated neurologic disease, the balance between the direct neuroprotective effect and the indirect angiogenesis/vessel normalizing effect should be evaluated in each model.

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