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Placental growth factor: a tissue modelling factor with therapeutic potentials in neurology?

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Abstract

Placental growth factor (PlGF) is an angiogenic factor that belongs to the vascular endothelial growth factor (VEGF) family. Besides its well known capacity to potentiate the angiogenic action of VEGF, PlGF also participates in inflammatory processes by attracting and activating monocytes; it plays therefore more specifically a role in pathological conditions. PlGF and its two receptors, VEGFR-1 and neuropilins (NRPs), are expressed in the brain and increase after experimental stroke, but their precise functions in the nervous system remain underexplored. In this review article, we summarize present knowledge on the role of PlGF in various nervous system disease processes. Given the available data, PlGF has neuroprotective and neurotrophic properties that make it an actor of considerable interest in the pathophysiology and potentially in the therapy of degenerative and traumatic brain or spinal cord diseases.

Key words: PlGF; neuroprotection; trauma; neurodegeneration; neuropilins; VEGFR-1.

Introduction

From Andreas Vesalius' anatomical drawings to current research, the number of studies highlighting the parallelism between vessel and nerve patterning is constantly increasing (1-8). The best known angiogenic factors belong to the vascular endothelial growth factor (VEGF) family. The VEGF family includes seven secreted glycoproteins, designated VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and placental growth factor (PlGF). They possess different physical and biological properties and act, as dimeric glycosylated proteins, through specific receptors: three protein–tyrosine kinase receptors (VEGFR-1, VEGFR-2, and VEGFR-3), present on most vascular endothelial cells and two non-protein kinase co-receptors, neuropilin-1 and neuropilin-2 (Fig. 1). Discovered first (9, 10), VEGF-A is the best characterized VEGF family member. VEGF-A mediates its effects by interacting with VEGFR-1 (also referred to as fms-like tyrosine kinase, Flt-1), and VEGFR-2 (also referred to as fetal liver kinase, Flk-1/KDR). In addition, $VEGF_{145}$ and $VEGF_{165}$, two isoforms of VEGF-A, can also bind to neuropilins (NRPs) (11, 12). Numerous reports have described the role of VEGF in the nervous system (NS), both during development (3, 6, 8, 13-17) and in neurogical disorders such as amyotrophic lateral sclerosis (18-28), Alzheimer's (29-31) or Parkinson's diseases (32, 33), brain ischemia (29, 34-43) or spinal cord injury (44, 46).

While VEGF is important under physiological and pathological conditions, PlGF (47), much less studied than VEGF-A, appears to be more specifically involved in pathological states (15). Although scientific data are scarce, we thought it timely to summarize our present knowledge on PlGF and the nervous system, as some of the pathological processes that are influenced by PlGF are relevant for the pathophysiology of various neurological disorders, and, possibly in the future, for their therapy. We will review the available information coming from direct studies of PlGF's functions in the nervous system from effects observed after its binding to its two receptors, VEGFR-1 and NRPs. The major findings on the known respective roles of VEGF and PlGF in experimental models of nervous system disorders are shown in table 1.

PlGF: biochemistry and functions

PlGF is a ~46 KDa dimeric glycoprotein that occurs in 3 isoforms in humans (131, 152 and 203 amino acids). Only one isoform, PlGF-2, is expressed in mice due to alternative mRNA splicing from a single gene (48). While PlGF was originally identified in the placenta (47), it has been detected

Table 1

VEGF and PlGF neurobiological effects in experimental models of neurological disorders.
Normal: beneficial effect; *Italic*: deleterious effect

Growth factor	Experimental models of neurological disorders	Neurobiological effect	References
VEGF	ALS (Mouse, SOD-1)	neuroprotection and neurotrophic action	19; 20; 22; 24; 27; 28
		reduction of astrogliosis and increased formation of neuromuscular junctions	26
	Cerebral ischemia (Rat, MCAO)	neuroprotection	37; 38; 42; 43
	Spinal cord injury (rat, clip injury)	increased angiogenesis, proliferation of glial progenitors, neuroprotection, tissue sparing, improvement of locomotor recovery	44; 45; 46
		exacerbation of lesion volume	86
	Parkinson (Rat, 6-OHDA)	neuroprotection, neovascularization and astroglial proliferation (anti-oxidant, GDNF source)	32; 33
	Alzheimer (Mouse, APP23Tg)	*maintenance of the chronic inflammatory response*	31
	Peripheral nerve injury	increased nerve regeneration, neurotrophic action, mitogenic activity on Schwann cells	82-84
PlGF	Cerebral ischemia	neuroprotection	50; 72
	Peripheral nerve injury	pro-inflammatory effects, mitogenic activity on Schwann cells	85

in several other organs including heart, lung, thyroid gland, skeletal muscles and more recently in brain. Numerous cell types, like endothelial cells, pericytes, macrophages, bone marrow cells, tumour cells, astrocytes and neurons, produce PlGF especially when they are activated or stressed like in hypoxia, inflammation or trauma (49-51).

PlGF potentiates the angiogenic effects of VEGF such as stimulation of endothelial cell proliferation and migration, increase of endothelial cell survival, induction of neoangiogenesis and vessel maturation (51). PlGF is also involved in inflammatory processes where it stimulates chemotaxis and activation of monocytes, and increases their secretion of pro-inflammatory cyto- and chemokines such as TNF-α, IL-1β and MCP-1 (52-54).

PlGF: a neuroprotective and neurotrophic factor

Contrasting with VEGF (55-59), there are only few reports on the potential neurotrophic/neuroprotective role of PlGF. The first study showing that PlGF might play an important role in the nervous system was performed by Beck *et al*. (50), who explored the expression pattern of NRPs, known to regulate both angiogenesis and neuronal axon guidance (3, 5, 8), in an experimental model of cerebral ischemia. Because NRPs have short intracellular segments without cytoplasmic signal transduction domains, they require an association with other receptors to be functional. These putative co-receptors include plexin A (52) as well as VEGFR-1 and VEGFR-2 (11, 13). Thus, in addition to NRPs, Beck *et al*. (50) also studied the expression of their co-receptors VEGFR-1 and 2, and their ligands VEGF-A and PlGF (61, 62). They demonstrated for the first time that both PlGF mRNA and its protein are expressed in neurons in the normal brain, but not in astrocytes, nor in vessels. They further showed that 3 days following a middle cerebral artery occlusion (MCAO), the PlGF transcript and protein are upregulated in vessels inside and around the infarcted area, as well as in neurons and astrocytes located at the ischemic border zone. These results were confirmed one year later in another report that assessed the expression of angiogenic genes after experimental brain ischemia (63). Thus, in addition to providing further support for the potential neurotrophic and neuroprotective roles of VEGF, these data suggested for the first time that PlGF may be implicated in pathological processes of the nervous system. This hypothesis was established later, notably thanks to Cheng *et al*. (64), who demonstrated that PlGF is able to exert an anti-chemorepulsive effect on axons through its NRP binding. NRPs are known to be

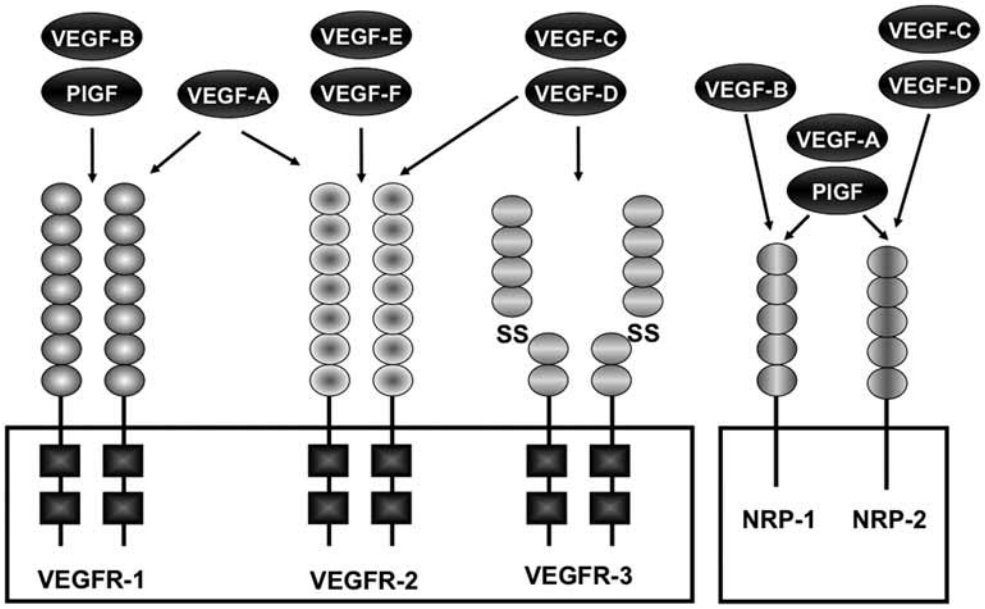

FIG. 1. — VEGF family members and receptors. The tyrosine kinase receptor VEGFR-1 binds VEGF-A, VEGF-B and PlGF with high affinity. VEGFR-2 binds VEGF-A, VEGF-C, VEGF-D, VEGF-E and VEGF-F. VEGFR-3 is a tyrosine kinase receptor with only six Ig-homology domains, which preferentially binds VEGF-C and VEGF-D. Neuropilin-1 and -2 (NRP-1 and NRP-2), the two non-tyrosine kinase-type co-receptors, respectively bind VEGF-A_{165}, VEGF-B and PLGF and VEGF-A_{165}, VEGF-C, VEGF-D and PLGF.

involved in axonal guidance during development, and the balance between their ligands, semaphorins and VEGF, plays a crucial role. On the one hand, NRPs, in conjunction with their co-receptors plexins, bind Sema3A and transduce repulsive signals to axons (60). On the other hand, when associated with VEGFR, NRPs bind VEGF and mediate attractive signal (13). Cheng *et al*. (64) analyzed neurite outgrowth in DRG explants and the effect of the NRP ligands, semaphorins, VEGF and PlGF, on the collapse of growth cones. Only $VEGF_{165}$ and PlGF were able to counteract the chemorepulsive effect of sema3A which supports a specific role for NRPs in mediating the neurotrophic actions of $VEGF_{165}$ and PlGF. More recently, a neuroprotective effect of PlGF was confirmed *in vitro* by a study showing that it promotes the survival of cultured primary cortical neurons under conditions of oxygen and glucose deprivation (65).

The neurotrophic and neuroprotective effects of PlGF, in addition to its already known angiogenic properties, are of great interest for the treatment of cerebral ischemia. The beneficial effects of cell therapy in experimental models of cerebral ischemia were shown in several studies (66-69). In particular, bone marrow derived mesenchymal stem cell (MSC) transplantations have beneficial neuroprotective and angiogenic effects, which are notably mediated by paracrine mechanisms (70). Among the various factors released by MSCs, VEGF and PlGF are indeed good candidates to explain these effects (71). This hypothesis is supported by a study (72) comparing the efficacy of systemically delivered human MSCs transfected or not with an adenoviral vector coding for PlGF in a rat MCAO model of cerebral ischemia. Transplantation of both cell types 3 hours after MCAO reduced the lesion size, induced angiogenesis and improved motor function, but greater effects were observed when rats were treated with the PlGF-transformed MSCs. These results suggest that PlGF might be an interesting candidate for neuroprotective treatment in ischemic stroke. Moreover, PlGF, probably via its binding to NRPs, could also be of therapeutic interest in neurodegenerative disorders where decreased vascularisation was described, or in spinal cord trauma where angiogenesis and neuroprotection are necessary to support and enhance axonal regeneration.

PlGF: a glial activator?

The glial response plays an important role after nervous injury. In the injured central nervous system, the proliferation and migration of astrocytes result in a gliotic scar that can exert both beneficial and detrimental effects. As a matter of fact, this glial scar isolates the injury site by re-establishing the glia limitans, thus restricting the propagation of secondary

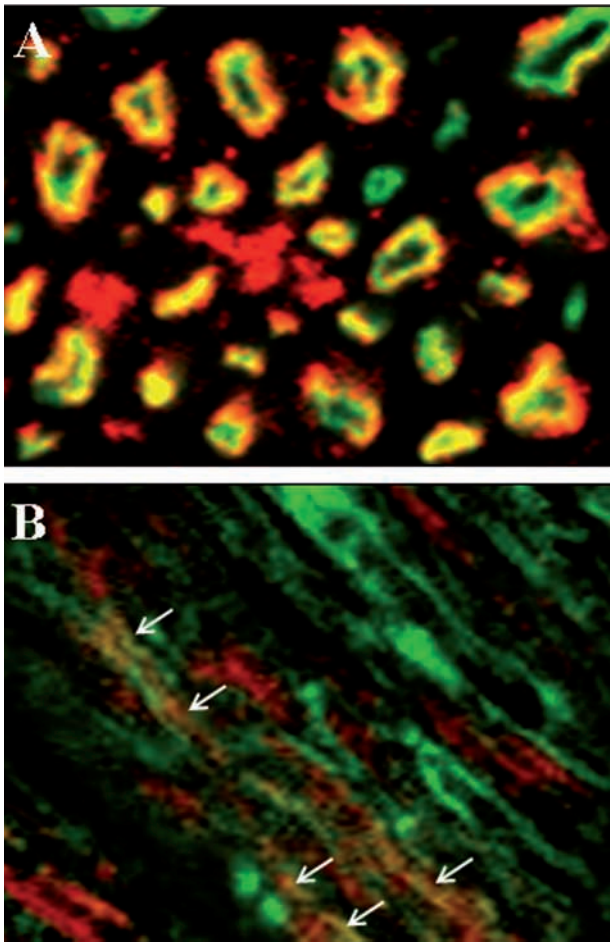

FIG. 2. — Double immunofluorescent stainings on normal and injured mouse sciatic nerve sections. PlGF (Rhodamine) and NF (A) or S100 (B) (FITC) double immunostainings, on cross-section of normal (A) and longitudinal section of injured (distal segment, B) sciatic nerves. In normal sciatic nerve, PlGF is found in axons underneath the axolemma (A). Schwann cells, which dedifferentiate and proliferate after loss of axonal contact, express PlGF from the first day after injury (B). Arrows point to Schwann cells expressing PlGF 24h after injury.

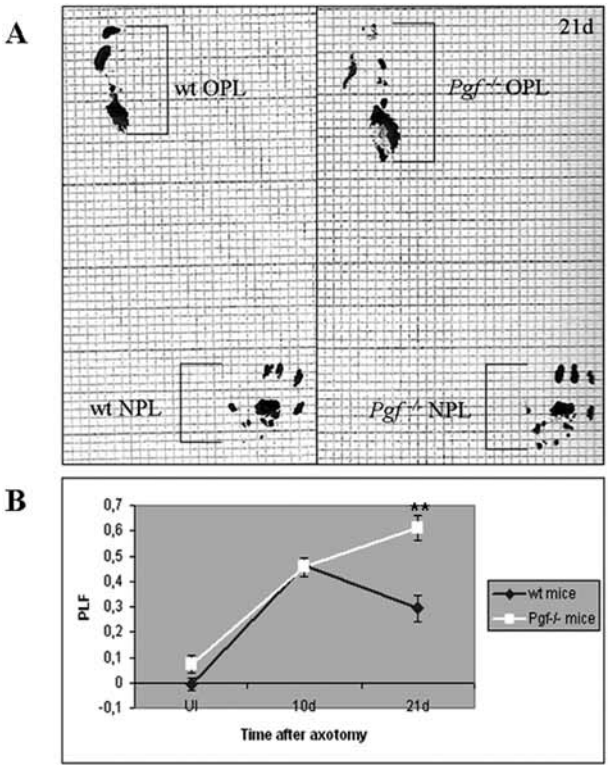

FIG. 3. — Functional recovery. (A) Representative footprints obtained from wt and *Pgf* $^{-/-}$ mice, 21 days after unilateral left sciatic nerve transection. Measurements of print length were made on the operated side (OPL) and the normal side (NPL). A mean of four values was then calculated for the OPL (xOPL) and NPL (xNPL), and a print-length factor (PLF) was calculated as follows: PLF = (xOPL – xNPL)/xNPL. (B) Graph showing the recovery of motor function after sciatic nerve transection determined by the PLF. During the first week following injury, PLF increases, indicating a marked disability in the injured paw. Later on, the PLF in wt mice progressively decreases, indicative of functional recovery. Worse recovery is evident in mice lacking PlGF compared to their wt controls.

lesions, but it also creates a physical and chemical barrier to axonal regeneration (73, 74). In central nervous system injury, the role of VEGF in the glial response has already been assessed. Several studies show that VEGF stimulates astroglial proliferation and migration via its flt-1 receptor, but also that it facilitates the expression of several growth factors, such as ciliary neurotrophic factor (CNTF) and basic fibroblast growth factor (bFGF) (75-77). By contrast, the influence of PlGF in the formation of the astroglial scar remains unknown. However, highlights have been recently brought by recent studies showing that the early growth response (Egr-1) factor, which regulates the astrocytic expression of phosphacan, a glial scar component, after experimental stroke (78), is a target gene for PlGF (79).

After peripheral nervous system injury, VEGF is expressed by Schwann cells (80, 81), stimulating their proliferation and migration (82-84) which is a crucial step for efficient axonal regeneration. Recently, we have published the first description of the expression and function of PlGF in the peripheral nervous system under normal physiological situation and after nerve injury (85). In the intact mouse sciatic nerve, PlGF is found in axons underneath the axolemma, while it is expressed by Schwann cells after axotomy (Fig. 2). Using transgenic knock-out mice for the PlGF gene (*Pgf* $^{-/-}$) and a multidisciplinary approach, we were able to demonstrate that PlGF plays a role in Wallerian degeneration: it stimulates proliferation and migration of Schwann

cells, as well as the inflammatory response by inducing MCP-1 expression and macrophage recruitment, essential for successful repair. Thus, PlGF depletion in *Pgf* $^{-/-}$ results in delayed axonal regeneration and impaired functional recovery (Fig. 3).

Conclusion

Currently, PlGF has received much less attention than VEGF-A as a potential treatment for neurological disorders. However, this careful analysis of the literature highlights the fact that the neuroprotective and neurotrophic effects of VEGF-A are mainly due to its binding to neuropilins and to a lesser extent to VEGFR-1, both of which being also PlGF receptors. It is only recently that specific studies have been devoted to PlGF demonstrating that it has its proper neuroprotective/neurotrophic properties.

A major drawback of the therapeutic use of VEGF-A in brain or spinal cord injury is increase in vascular permeability and oedema formation (87) because of its pro-angiogenic properties (9, 86). The effect of PlGF on vascular permeability remains disputed. Some reports have shown that only VEGF-A analogues activating VEGFR-2 are able to increase vascular permeability (88, 89). The lack of such an effect for PlGF could thus make it a more appropriate candidate than VEGF-A for neurological treatment. However, experiments using mutant mice in which PlGF is under- or over-expressed have respectively revealed a decrease (90) or an increase (91, 92) of the vascular permeability in pathological conditions. One explanation, according to Carmeliet *et al*. (93) is that the PlGF-induced increase in vascular permeability is an indirect effect, consequence of the displacement of VEGF-A from VEGFR-1 to VEGFR-2, as PlGF competes with VEGF-A for VEGFR-1 in pathological conditions.

Taken together, VEGF and PlGF, despite proven experimental advantages, could be double-sided swords as neuroprotective/neurotrophic treatments for ischemic, degenerative or traumatic neurological disorders. In future therapeutic studies, it might be worthwhile to explore the effect of a combination of PlGF to induce beneficial tissue modelling effects and a VEGFR-2 inhibitor to block the VEGF-dependent angiogenic effect.

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