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Review

GDNF, a neuron-derived factor upregulated in glial cells during disease.

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Abstract: In healthy adult brain, glial cell line-derived neurotrophic factor (GDNF) is exclusively expressed by neurons and in some instances, it has furthermore been shown to derive from a single neuronal subpopulation. Secreted GDNF acts in a paracrine fashion by forming a complex with GDNF family receptor α1 (GFRα1) which is mainly expressed by neurons and can act in *cis* as a membrane-bound or in *trans* as a soluble factor. The GDNF/GFRα1 complex signals through interaction with RET ("rearranged during transfection") or with a lower affinity with neural cell adhesion molecule (NCAM). GDNF can also signal independently from GFRα1 via interaction with syndecan-3. RET being expressed by neurons involved in several pathways: nigro-striatal dopaminergic neurons, motor neurons, enteric neurons, sensory neurons, etc. could be the main determinant of the specificity of GDNF pro-survival effect. In injured brain, de novo expression of GDNF occurs in glial cells. Neuroinflammation has been reported to induce GDNF expression in activated astrocytes and microglia, infiltrating macrophages, nestin-positive neural stem cells and neuron/glia (NG2) progenitors. This disease-related GDNF overexpression can be either beneficial or detrimental depending on the localization in the brain and the level and duration of glial cells activation. Some reports also describe upregulation of RET and GFRα1 in glial cells, suggesting that GDNF could modulate neuroinflammation.

Keywords: GDNF; microglia; astrocyte; neuroinflammation; RET; GFRα1; gene therapy; Parkinson's disease

1. Introduction

Glial cell line-derived neurotrophic factor (GDNF) has been isolated from conditioned media of a rat glioma cell line on the basis of its trophic activity towards primary cultures of dopaminergic neurons [1].

Following administration of GDNF family ligands (GFL) in animals, neurorestorative effects have been demonstrated in models of several neurological diseases [2-4]. However, in cases of high doses and long-term administration, aberrant sprouting and negative feedback effects on neurotransmitter homeostasis have been observed [5-7].

Two members of the GFL, GDNF and Neurturin, have been evaluated in clinical trials for Parkinson's disease (PD) [8,9]. Although positron-emission tomography (PET) scan imaging has evidenced clear functional improvements [10,11] and post-mortem analysis have demonstrated neuronal sprouting at the site of delivery [12], clinical outcomes have been disappointing. Therefore, a better knowledge of GDNF mechanism of action *in vivo* in complex neuronal circuits is urgently needed in order to revisit the clinical paradigms.

GDNF is mainly expressed during development and is involved in neuronal specification [13-16]. In healthy adult brain, GDNF expression decreases and is restricted to some regions: cortex,

hippocampus, striatum, substantia nigra[1], thalamus, cerebellum and spinal cord [17-19]. GDNF is a secreted factor [20] mainly expressed by neurons and acts mainly on neurons expressing RET ("rearranged during transfection") receptor. In few instances, it has furthermore been demonstrated to derive from a specific neuronal subpopulation [17,21]. For example, in the striatum, only interneurons [22] and among them, mainly the parvalbumin-expressing interneurons [21,23] express GDNF.

Thus, in healthy rodent brain, GDNF appears to be a neuron-derived rather than a glia-derived neurotrophic factor. However, the pattern of GDNF expression is different in diseased brain. Indeed, de novo GDNF expression in glial cells has been described in numerous models of diseases, usually concomitantly with neuroinflammation.

In this review, we will discuss the dual role of GDNF upregulation in glial cells during neurodegeneration and repair. Other reviews covering the expression of neurotrophic factors by glial cells already exist [24,25]. Contradictory conclusions often arise from data obtained *in vivo* and in *in vitro* (see discussion in reference [26]). In the present review, for clarity, we will focus on the expression of GDNF and its receptors in the nervous system *in vivo* except when mentioned for some aspects which have only been addressed *in vitro.*

2. Glial cells express GDNF during disease

GDNF is expressed by neurons in developing [27,28] and adult nervous system [17,19,21,27,28]. The expression patterns of GDNF have been confirmed using genetically modified mice [29] and does not match astrocytes distribution as revealed using anti-Glial Fibrillary Acid Protein (GFAP) antibodies [17]. Therefore, the name given to this neurotrophic factor can be misleading. Contrasting with most publications that failed to demonstrate GDNF glial expression in the healthy brain, Ubhi et al. have shown that, a small proportion (around 6%) of GDNF-expressing cells are glial [30].

Numerous studies have evidenced de novo expression of GDNF by glial cells in injured brain (see Table 1). In disease models, neuroinflammation can upregulate GDNF expression in activated astrocytes [31-38], microglia and infiltrating macrophages [31,34,39-43], nestin/GFAP-positive neural stem cells [44] and ionized calcium-binding adapter molecule 1 (Iba1)-positive neuron/glia NG2 progenitors [45]. This disease-related GDNF overexpression can be either beneficial [45,46] or detrimental depending on the age of the animal [47], the length of the neuroinflammatory response [34,48] and the type of glial cells activated [26].

GDNF produced by activated microglia/macrophages can lead to repair of CNS injuries. After striatal mechanical injury [39,40] and spinal cord injury [41], activated microglia and macrophages express GDNF, thereby inducing axonal sprouting and locomotor improvements. In the 6-hydroxydopamine (6-OHDA) PD model, around 60% of the surviving tyrosine hydroxylase (TH)-positive neurons are located near NG2 cells that express GDNF [45]. In the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) PD model, cinnamon-induced neuroprotection was shown to be mediated by astrocytic GDNF overexpression in substantia nigra [37].

However, glial GDNF overexpression could be a double-edge sword. Indeed, after a mechanical injury of the striatum, GDNF-induced axonal sprouting failed to crossover the wound edge [39]. Such a dual effect of local GDNF overexpression was also observed in a spinal cord repair paradigm in which a transplanted nerve root genetically modified with a lentiviral vector expressing GDNF in Schwann cells, stimulated the regeneration of motor neuron axons locally but not beyond the lesion [6]. It should be noted however, that the deleterious effect of local GDNF overexpression is not limited to glia since long-lasting GDNF overexpression by neurons can also lead to aberrant sprouting in case of ectopic localization [5,49].

[1] GDNF mRNA expression in the substantia nigra dopaminergic neurons has been observed in reference 21 but not in reference 23. This discrepancy could be explained by differences between the probes used for in situ hybridization, the sensitivity of the method (digoxygenin-labeled versus radiolabeled probes) and the sex of the animals (female versus male).

Table 1. Non-neuronal GDNF expression during disease .

Cell type	Disease model	Methods used	References
Macrophages/ microglia	Striatal mechanical injury	ISH + Immunohistochemestry	[39,40,42]
	Experimental autoimmune neuritis	Double Immunofluorescence	[43]
	LPS-induced inflammation	Double Immunofluorescence	[34,41]
Astrocytes	Quinolic acid lesion	Double Immunofluorescence	[32,33]
	LPS-induced inflammation	Double Immunofluorescence	[34]
	6-OHDA	ISH + Immunofluorescence	[35]
		Double Immunofluorescence	[36]
	MPTP	Double Immunofluorescence	[37]
	Spinal cord ischemia	Double Immunofluorescence	[38]
Neural precursors	MPTP	Double Immunofluorescence	[44]
NG2 cells	6-OHDA	Double Immunofluorescence	[45]

ISH, *in situ* hybridization; 6-OHDA, 6-hydroxydopamine; LPS, lipopolysaccharide: MPTP; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

Transgenic mice expressing GDNF either from the endogenous locus, or from a GFAP promoter, revealed that astrocytic-derived GDNF overexpression is responsible for TH downregulation, decreased dopaminergic neurotransmission and motor deficits [26]. On the other hand, GDNF overexpression from the native locus, i.e. in neurons, leads to an increase number of dopaminergic neurons in the substantia nigra, increased dopamine transporter (DAT) activity, increased dopamine neurotransmission and improved motor behavior [23]. Taken together, these results suggest that a prolonged astrocytic GDNF overexpression is harmful whereas, a prolonged neuronal overexpression of GDNF is rather beneficial for the dopaminergic system.

3. Glial cells express GDNF receptors during disease

GDNF forms a complex with its primary receptor, GFRα1, which can be membrane-bound or released in a soluble form [50,51][2]. The GDNF-GFRα1 complex then binds RET [19,52] present on neurons cell bodies and terminals of several different pathways such as nigro-striatal dopaminergic neurons [53,54], spinal motor neurons [55], noradrenergic neurons of the locus coeruleus [56], enteric neurons [57] or sensory neurons [58]. The GDNF-GFRα1 complex can also bind to and induce signaling through the neural adhesion molecule, NCAM [59-62]. GDNF can furthermore directly interact with the heparin sulfate proteoglycan, syndecan-3 [63-65]. Interestingly, GDNF binding to heparan sulfate has been shown to be beneficial for the protection of dopaminergic neurons in the 6-OHDA rat model of PD [66].

Upregulation of GFRα1 and RET has been reported in glial cells in pathological conditions [32,33,67-69] (see Table 2).

RET was shown to be expressed in microglia in brain tissue from patients with PD but not from healthy controls [67]. RET and its phosphorylated form (pRET) were also gradually increased in microglia during disease progression in a transgenic mice model of amyotrophic lateral sclerosis (ALS) [69,70]. In parallel, RET expression was decreased in motor neurons. These data could indicate that motor neurons die due to a lack of response to neurotrophic factors or to an excess of neurotoxic compounds derived from activated microglia.

On the other hand, increased neuronal survival concomitant with RET activation in microglia was described in hippocampal slices treated with the excitotoxin, N-methyl-D-aspartate (NMDA) [68].

Excitatory amino acids induced GDNF and GFRα1 but not RET de novo expression by astrocytes in the striatum following treatment with quinolinate or kainate [32,33]. Similarly, after a mechanical lesion of the spinal cord of adult rats, GDNF and GFRα1 were upregulated in astrocytes [71].

[2] The existence of a soluble form of GFRα1 has been shown in primary cultures of neurons.

Table 2. Upregulation of GDNF receptors in activated glial cells.

Receptor	Cell type	Disease/lesion	Methods	Reference
GFRα1	Astrocytes	Rat striatum treated with quinolinic acid.	Double immunofluorescence	[33]
		Rat striatum treated with quinolinic acid or kainic acid.	GFRα1 immunoreactivity, morphology	[32]
		Spinal cord mechanical injury.	GFRα1 immunoreactivity, localization in white matter, morphology.	[71]
RET	Microglia	Human PD and aging.	Single immunohistochemistry, morphology	[67]
RET, pRET	Microglia	ALS transgenic mice.	Double immunofluorescence.	[69]
pRET	Microglia?	Rat hippocampal slices treated with NMDA and exogenous GDNF.	Immunofluorescence combined with isolectin IB4[3]	[68]

ALS, amyotrophic lateral sclerosis; NMDA, N-methyl-D-aspartate; pRET, phosphorylated RET.

4. Conclusions and Further Prospects

The role of GDNF in healthy and diseased brain is contrasting. From a mainly neuron-derived secretion and a specific neurotrophic action during development and to a reduced extent in the adult brain, it can turn into a glia-derived factor which can rescue neurons but also possibly support glial cells activation during neuroinflammation [33,70] (See Figure 1). However, long-term excessive or ectopic GDNF-mediated neurotrophic activity has been shown to result in aberrant sprouting [5,39] or axon entrapment [4,6].

Activated microglia and astrocytes exist in different states which can be neuroprotective [39-41,74,75] or neurotoxic [48,76]. Numerous studies suggest that acute neuroinflammation resulting in phagocytosis of dead cells debris is beneficial. In contrast, continuous neuroinflammation becomes deleterious due to high levels of cytokines, reactive oxygen and nitrogen species which are toxic to neurons [77]. Attenuation of the sustained neuroinflammatory response actually increases neuron survival [70].

Neuroinflammation has been shown to induce de novo expression of GDNF in glial cells [34,41,78] possibly via NF-κB-responsive elements present in the GDNF promoter [79-81]. In turn, GDNF, after binding to GFRα1, also upregulated in disease, could possibly increase survival of activated microglia through activation of RET signaling. It is therefore tempting to hypothesize that in situations where the neuroinflammatory process becomes uncontrollable, disease-induced GDNF could contribute to perpetuate microglial activation. Astrocytes were shown to express GDNF and GFRα1 but not RET in pathological conditions [32]. However, since GFRα1 is a diffusible factor, GDNF could induce a trophic signaling in other cell types expressing RET or NCAM.

GDNF and Neurturin have been proposed as therapeutic agents for PD [8-10]. Although functional improvements were observed by PET scan imaging [10,11,82] and fiber sprouting was observed in post-mortem samples [12,83], the clinical benefits were very modest.

The emerging picture of the deleterious effect of long-term uncontrolled GDNF overexpression suggests that the clinical benefits could have been reduced by aberrant neurotrophic activity perturbing bona fide neuronal circuit repair.

In gene therapy paradigms using GFL [8,84], our assumption is that transgene expression should be controlled in order to avoid aberrant sprouting and perpetuation of neuroinflammatory processes which can become deleterious. Clinically-acceptable genetic switches are becoming available and could improve the outcome of future clinical trials with GFL [85-92].

[3] IB4, an isolectin, widely used to label microglial cells *in vitro* [72] was shown to directly interact with RET an observation which questions the identification of IB4-labeled cells *in vivo* [73].

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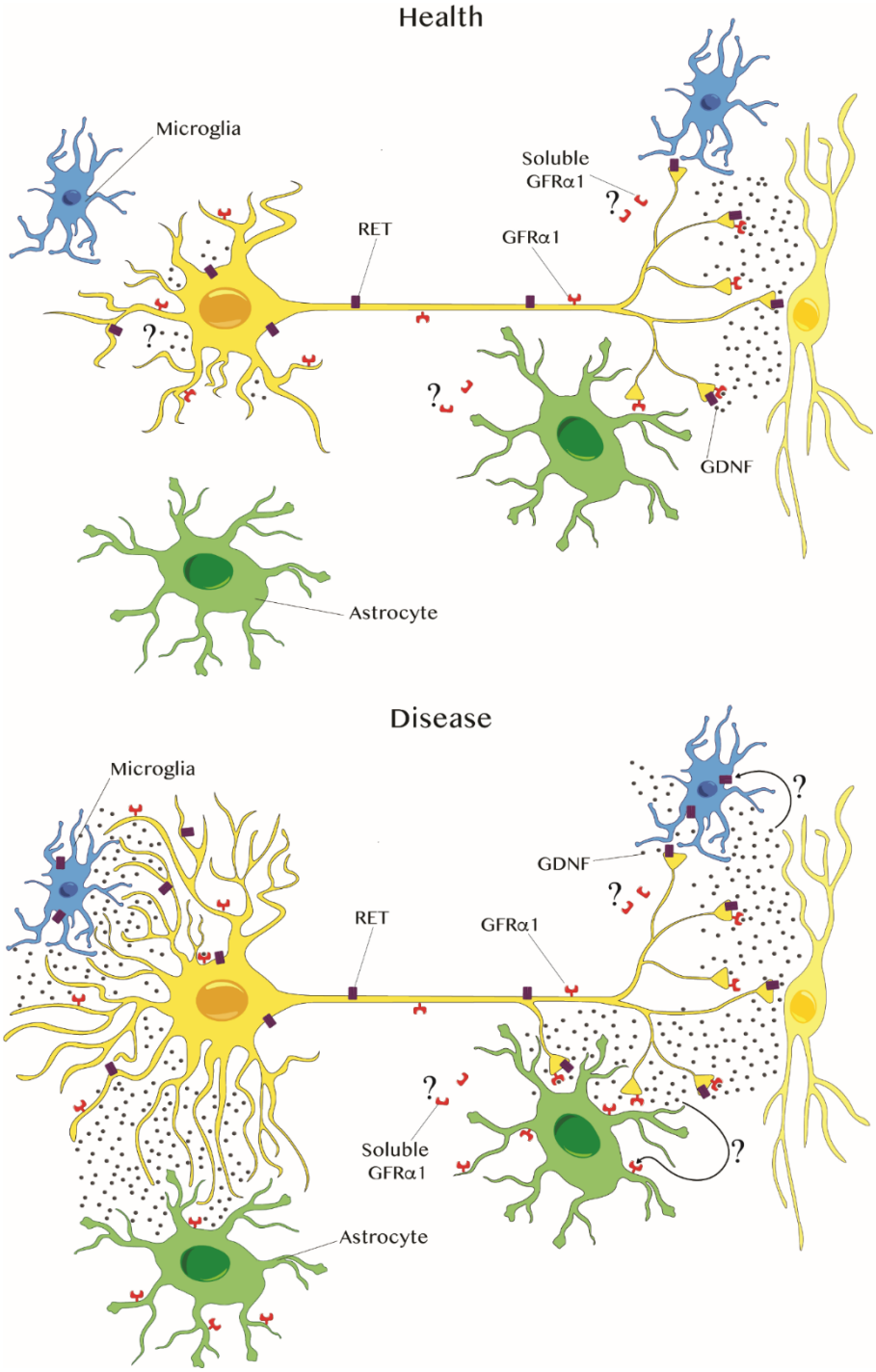

Figure 1. GDNF and GDNF receptors in healthy brain and during disease. Upper panel: In healthy nervous system, GDNF expression is mainly neuronal. GDNF forms a complex with GFRα1 present in the neuronal membrane. This complex binds to RET, a transmembrane receptor, triggering an intracellular signaling cascade that promotes survival. A few *in vitro* studies have reported that GFRα1 exists in a soluble form, suggesting that GDNF can have broader effects. However, these data lack *in vivo* confirmation. Lower panel: Several studies report that during disease, glial cells can also express GDNF. It remains unclear whether the glial expression is beneficial or detrimental. In fact, GDNF expression can promote survival and axonal growth but a sustained GDNF overexpression or ectotopic GDNF expression can lead to aberrant sprouting. In pathological cases, microglia express RET but not GFRα1 suggesting that RET signaling may occur in a GDNF-independent manner or through a GFRα1 soluble form. In disease conditions, GFRα1 is upregulated in astrocytes but there is no evidence of RET expression. Further investigation is required to establish the effects of GDNF-GFRα1 astrocytic interaction.

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