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Progranulin does not affect motor neuron degeneration in mutant *SOD1* mice and rats

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ABSTRACT

Motor neuron degeneration in amyotrophic lateral sclerosis (ALS) is familial in 10% of patients, with mutations in *SOD1* and *C9orf72* being the most frequent cause. There is convincing evidence for overlap between ALS and frontotemporal lobar degeneration at the genetic, pathological, and clinical level. Null mutations in progranulin (*PGRN*) are a frequent cause of familial frontotemporal lobar degeneration. *PGRN* exerts neurotrophic properties on motor neurons in vitro and in vivo. We therefore examined whether *PGRN* could affect disease progression in mutant *SOD1* mice and rats, both established models for ALS. Overexpression of *PGRN* in mice and intracerebroventricular delivery of *PGRN* in rats did not affect onset or progression of motor neuron degeneration.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are related and sometimes overlapping neurodegenerative disorders, often characterized by ubiquitin- and TAR DNA-binding protein 43 (TDP43) positive inclusions in the brain and spinal cord. Some genetic forms of ALS, FTLD, or ALS-FTLD have a common cause, such as mutations in *C9orf72*, or rarely in *VCP*, *TARDBP*, *CHMP2B*, *UBQLN2*, or the gene encoding *PGRN*. Mutations in other genes are more specifically associated with 1 phenotype. Mutations in *SOD1* are a frequent cause of pure ALS and several established models of mutant *SOD1* (mSOD1)-induced motor neuron degeneration exist. Progranulin (*PGRN*) is a widely expressed pleiotropic growth factor, with neurotrophic properties in vitro and in vivo and it plays a role in neuroinflammation. Loss of function mutations in the gene encoding *PGRN* are a frequent cause of FTLD, rarely in conjunction with ALS. The resulting haploinsufficiency of *PGRN* is likely to bring about neurodegeneration in FTLD, but has not been observed in other forms of ALS and/or FTLD. Given the neurotrophic properties of *PGRN* and the shortage of *PGRN* involved in 1 form of ALS and/or FTLD, *PGRN* is a potential

therapeutic target for ALS and/or FTLD. We therefore examined whether *PGRN* overexpression or intracerebroventricular (ICV) delivery could affect disease progression in mSOD1 mice and rats, both established models for ALS.

2. Methods

Conditional *PGRN* mice were generated by expression of human *PGRN* (hPGRN) cDNA preceded by a floxed Neomycine stop cassette in the *ROSA26* locus. After crossbreeding these mice to PGK-Cre mice, ubiquitous and constitutive *PGRN* overexpression mice were obtained. *PGRN* messenger RNA (mRNA) and protein overexpression was demonstrated using quantitative polymerase chain reaction and enzyme-linked immunosorbent assay. These mice were crossbred to mSOD1^{G93A} mice. mSOD1^{G93A} rats were treated with ICV delivery of recombinant *PGRN*, as previously described. All methods and results are described in detail in the [Supplementary data](#).

3. Results

Transgenic mice that overexpress *PGRN* showed ubiquitous overexpression of *PGRN*, including overexpression in brain and spinal cord, at the mRNA and protein level. *PGRN* was expressed in various cell types, as shown using quantitative polymerase chain reaction on cultured (motor and cortical) neurons and glial cells and

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by immunostainings in vivo. Crossbreeding of PGRN overexpression mice to mSOD1^{G93A} mice did not affect the onset of disease (as determined using the paw grip endurance test and rotarod) or the survival. In addition, quantification of denervated neuromuscular junctions of the gastrocnemius muscle did not show protection. As a second strategy to test the therapeutic potential of PGRN, mSOD1^{G93A} rats were treated with ICV PGRN using miniosmotic pumps connected to an ICV catheter. This resulted in elevated hPGRN levels in cerebrospinal fluid, measured using enzyme-linked immunosorbent assay, but this treatment also failed to show a positive effect on the onset of motor neuron degeneration or survival.

4. Discussion

We tested the therapeutic potential of PGRN on motor neuron degeneration induced by mSOD1. Despite the neurotrophic effects of PGRN on cultured motor neurons, overexpression of PGRN or ICV treatment of PGRN did not affect the onset or progression of motor neuron degeneration in mSOD1^{G93A} rodent models.

These negative results were obtained using 2 complementary strategies: a crossbreeding experiment with overexpression in all cell types, in combination with an ICV delivery experiment, which is directly translatable to patients.

Although negative, these results are in line with what we previously observed in a zebrafish model of mSOD1-induced axonopathy. In zebrafish, hPGRN overexpression alleviated mutant TDP43-induced axonopathy, but had no effect on mSOD1-induced axonopathy. ALS patients with *SOD1* mutations do not show

ubiquitinated TDP43-inclusions, which are present in most of ALS cases. This suggests a divergent disease mechanism for SOD1 and TDP43 and suggests that PGRN might only have beneficial effects in TDP43 proteinopathies.

Disclosure statement

The authors have no financial or other conflicts of interest.

All experiments on rodents were approved by the local ethical committee of the University of Leuven, Belgium.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2013.03.027>.