EPHA4 is a disease modifier of amyotrophic lateral sclerosis in animal models and in humans

Call for projects 2009
Grant: 336 000 €
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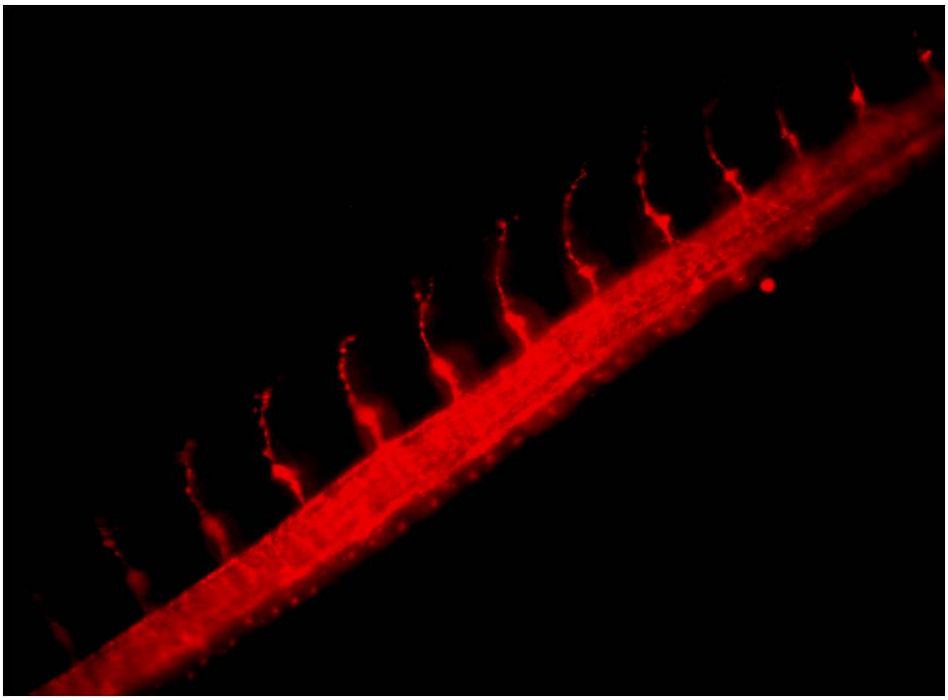
In the project, investigators at the laboratory for Neurobiology of the Vesalius Research Center at the Flemish Institute for Biotechnology (University of Leuven, Belgium) used a zebrafish model for ALS in order to identify genes of which the knockdown rescued the phenotype induced by mutant SOD1, an ALS-causing protein. One such gene was found to be the gene encoding EphA4, a receptor in the ephrin repellent system. To confirm this zebrafish finding in larger vertebrate models, they genetically deleted the EphA4 gene from the mutant SOD1 mouse and found this to attenuate the motor neuron degeneration in this mouse model for ALS. Interestingly, not only genetic but also pharmacologic inhibition of EphA4 rescued the zebrafish phenotype, and slowed disease in the mutant SOD1 rodent model. To investigate whether these findings in laboratory models also held in patients with ALS, they quantified EphA4 expression in ALS patients and controls, and found it to correlate with disease severity as defined by age at onset and duration of disease. Furthermore, they identified two patients with a loss-of-function mutation in the EphA4 gene with unusually long survival. All these data suggest that low expression of EphA4 attenuates disease severity, and thus that EphA4 is a modifier of ALS in animal models and patients.

The investigators then studied the mechanism through this receptor may affect the disease process. They found that vulnerable motor neurons express high and more resistant motor neurons express low levels of it. Furthermore, decreasing the EphA4 expression in motor neurons enhanced their regenerating capacity.

Of notice, EphA4 knockdown also rescued the phenotype induced by mutant TDP43, another cause of ALS, and of SMN knockdown, the cause of another motor neuron disease, spinal muscular atrophy, suggesting that the modifying effect of EphA4 may be generic to motor neuron degeneration, and possibly to other forms of neurodegeneration.

The results of this research imply that EphA4 is a therapeutic target in ALS. As this is a receptor, it can be approached using both small compounds and peptidergic antagonists, as already shown in the zebrafish and mutant SOD1 rat, resp. This may lead to the development of EphA4 antagonists for the treatment of ALS and maybe other neurodegenerative disorders.



Zebrafish embryo: spinal cord and motor neurons axons.

TRANSLATIONAL RESEARCH INTO THE ROLE OF THE EPHRIN SYSTEM IN THE PATHOGENESIS OF AMYOTROPHIC LATERAL SCLEROSIS

P. Van Damme, Belgique
grant € 336 500

Disease modifying genes are thought to modify the phenotype induced by mutant proteins that cause familial ALS, and to represent risk or protective factors for sporadic ALS. It is important to identify the cellular mechanisms through which these factors act, as they are possible targets for therapeutic intervention.

In order to identify genes that protect against mutant SOD1-induced neurodegeneration, we established a zebrafish model that allows a morpholino-based genetic screen. In this model, we found that the motor axonopathy induced by the SOD1A4V mutant is completely and dose-dependently reversed by knocking down an ephrin receptor. Ephrin receptors and their ligands, the ephrins, form a ubiquitously expressed intercellular communication system with bidirectional signaling activity. The possible involvement of this system in ALS has not been studied.

The present project aims to study the role of ephrins in the pathogenesis of ALS, and to explore the possible therapeutic implications of interfering with it.

We will investigate the localization of the ephrin receptor and its ligands in the spinal cord of the non-transgenic, wildtype SOD1 and SOD1G93A mouse, and will study its role in the motor neuron degeneration of the SOD1G93A mouse by deleting it from the SOD1G93A mouse. Furthermore, we will investigate whether its knockdown also resues the axonopathy induced by mutant TDP-43, which has recently been identified as a cause of familial ALS. In addition, we will explore the effect of antagonists in SOD1G93A models and will study the possible association between single nucleotide polymorphisms in the relevant human ephrin receptor gene and the occurrence of ALS

TEAM

Philip Van Damme, Vincent Thijs and Wim Robberecht are neurologists, working in the Neurology Department of the University Hospital Leuven (www.neurology-kuleuven.be), with main interest in neuromuscular disorders and genetics, and performing research into the pathogenesis of neurodegeneration in the Vesalius Research Center, VIB, Leuven (www.vrc-lab.be) and the Department of Experimental Neurology, K.U. Leuven. Diether Lambrechts is running the gene-

tics facility at VRC. Wim Robberecht is head of the Department of Neurology and Experimental Neurology.

Angela Laird is a postdoctoral fellow, Annelies Van Hoecke and Lies Schoonaert are PhD students and Mieke Timmers a lab technician, all working on this project. The main focus of the lab is the study of the pathogenesis of amyotrophic lateral sclerosis using genetic, transgenic animal and cellular approaches in order to develop new therapeutic strategies.