Novel gene therapy approach for motoneuron disease through broad delivery of death signalling inhibitors by astrocytes

Call for projects 2009
Grant: 210 000 €
Project Duration: 3 years

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A novel motoneuron-specific cell death pathway

Death pathways restricted to specific classes of neurons might explain the selectivity of neuronal loss in neurodegenerative diseases, such as the loss of motoneurons in ALS. A molecule called LIGHT (TNFRSF14), a member of the TNF superfamily is expressed by immune cell types and plays an important role in innate and adaptive immunity. We have unravelled a novel motoneuron selective death pathway triggered by LIGHT through the activation of the receptor LT-βR. Furthermore interferon gamma (IFNγ), selectively induces death of motoneurons through the activation of LT-βR by LIGHT. Importantly, we found that purified motoneurons are rescued from the neurotoxicity of astrocytes expressing ALS-linked mutant SOD1 by blocking LIGHT/LT-βR interaction and by antagonizing IFNγ. In the ALS mouse model, levels of both IFNγ and LIGHT are significantly elevated in the spinal cord. In addition, we found that genetic ablation of *Light* delays progression but not onset of the disease and increases lifespan of ALS mice. This evidence confirms the involvement of the LIGHT pathway in motoneuron degeneration.

Pertinence of the IFNγ and LIGHT pathway in sporadic ALS

We found that IFNγ and LIGHT levels are also significantly upregulated in spinal cords of patients with sporadic ALS. Remarkably, IFNγ was abondandly present in motoneurons and in astrocytes as well as in cells reminiscent of invading immune cells. LIGHT was mainly found in motoneurons. Strikingly, levels of IFNγ are increased in the cerebrospinal fluid and serum of ALS patients. Collectively, this evidence supports a potential role of IFNγ, which can arise from astrocytes, immune cells or motoneurons themselves in the pathogenic process.

A gene therapy approach to ALS

To next evaluate the therapeutic potential of interfering with IFNγ/LIGHT and FasL death pathways we developed a gene therapy approach based on the delivery of recombinant dominant negative receptors by means of adeno-associated-viral vectors (AAV). Chimeric dominant negative receptors against IFNγ, LIGHT and/or FasL were constructed. The delivery of these designed receptors to primary cultured motoneurons rescued them from death-inducing concentrations of IFNγ, LIGHT and/or FasL. An important aspect of therapeutic approaches for motoneuron diseases is the delivery of therapeutic molecules along the entire central nervous system. Intracerebroventricular (i.c.v) injection of AAV9 in newborn pups allows infecting mainly astrocytes in the brain and along cervical, thoracic and lumbar regions of the spinal cord. Since astrocytes are

an attractive platform to express inhibitory receptors in the extracellular milieu of motoneurons, we based our study on this viral serotype and used an astrocyte-specific promoter. We showed that i.c.v injection of AAV9 leads to a stable expression of the transgenes. We are currently evaluating the therapeutic benefits of the expression of these designed receptor in a first cohort of SOD1^{G93A} mice.

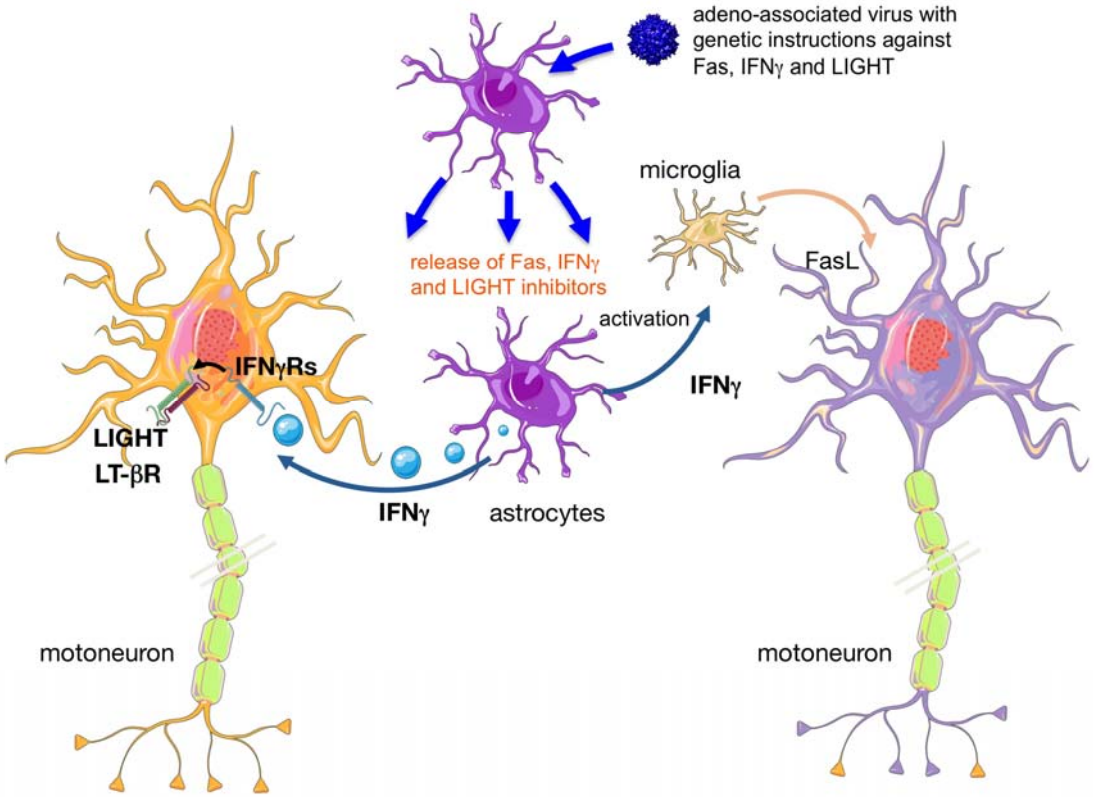
Publications/manuscripts acknowledging the Thierry Latran Foundation

Aebischer, J., Cassina, P., Otsmane, B., Moumen, A., Seilhean, D., Meininger, V., Barbeito, L, Pettmann, B. and Raoul, C. (2011) IFNγ triggers a LIGHT-dependent selective death of motoneurons contributing to the non-cell-autonomous effects of mutant SOD1. **Cell Death Diff**. 18, 754-768.

Aebischer, J., Moumen, A., Sazdovitch, V., Seilhean, D., Meininger, V. and Raoul, C. Elevated levels of IFNγ and LIGHT in the spinal cord of sporadic amyotrophic lateral sclerosis patients. **Eur J Neurol.** 2012; 19(5):752-9.

Dirren, E., Towne, C.L., Setola V., Redmond, D.E. Jr, Schneider B.L. and Aebischer P. Intracerebroventricular injection of AAV6 and AAV9 vectors for cell type specific transgene expression in the spinal cord. *Submitted*.

Figure: A novel mechanism of motoneuron death and its therapeutic potential



NOVEL GENE THERAPY APPROACH FOR MOTONEURON DISEASE THROUGH BROAD DELIVERY OF DEATH SIGNALLING INHIBITORS BY ASTROCYTES

Patrick Aebischer(1) and Cédric Raoul(2)
Grant : € 210 000

ALS is characterized by the selective loss of motoneurons in the brain and spinal cord. Accumulating evidence suggest that neighbouring cells of motoneurons, namely astrocytes, actively participate to the pathogenesis of ALS, by releasing factors that selectively kill motoneurons. The identification of motoneuron-restricted death pathways triggered by death receptors might explain this selective vulnerability of motoneurons to toxic action of astrocytes. Astrocytes, by being the most abundant cells of the nervous system and intimately associated with motoneurons, are pertinent cellular platforms to secrete protective molecules. Recently, vascular delivery of adeno-associated viral vectors has been shown to transduce astrocytes throughout the brain and spinal cord. Nevertheless, the conversion of astrocytes into therapeutic supports for upper and lower motoneurons needs to be validated.

This collaborative project aims at evaluating an innovative gene therapy approach to deliver to the broadest population of motoneurons, including upper and lower motoneurons, diffusible inhibitors of death signalling in pre-clinical model of motoneuron disease. In addition, a genetic approach and an alternative route of delivery will be developed in parallel to further strengthen our therapeutic approach and hopefully open new perspectives for ALS therapies.

TEAM

This project is based on a long and strong collaboration that combine expertise on death receptor signalling and gene therapy approaches for neurodegenerative diseases

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