Research Project Proposal
Academic year 2018-2019

Project Nº 46

Title: *Modulation of the Unfolded Protein Response in Motoneurons: A new Gene Therapy for Acute Lateral Sclerosis?*

Department/ Laboratory *Laboratories 4.05 and 4.08, Department of gene therapy and regulation of gne expression*

Director 1 *Tomas Aragón*
Contact: *taragon@unav.es*
Codirector: GloriaGonzález-Aseguinolaza
Contact: *ggasegui@unav.es*

Summary

The work offer to the master student will be related to the following specific aims:

The working hypothesis of this project is that targeting of the genes involved in the UPR in motoneurons could enhance the survival of this neuronal subset in murine ALS models based on the expression of mutant fALS alleles. As mentioned above this idea is supported by the notion that the genetic or pharmacological targeting of PERK in neurons improves the symptomatology of model animals. In this project we will:

1. Design CRISPR Cas9 constructs to efficiently target the three UPR mechanisms, as well as the Integrated Stress Response. Specifically we will optimize silencing guide RNAs to target IRE1, PERK, ATF6, XBP1, ATF4, PKR, GCN2 and HRI.
2. We will generate recombinant AAVs expressing GFP under the control of different motoneuron promoters (for instance Hb9, UCHL1, Smn). With these vectors, we will identify which promoter system provides the most efficient, selective expression of GFP in motoneurons.
3. Then, we will generate recombinant AAVs expressing the *Staphylococcus aureus* Cas9 protein under the control of our motoneuron promoter of choice and the UPR/ISR guide RNAs designed earlier. We may also develop vectors to silence the transgenic human SOD1 G93A allele of our ALS mouse model, to demonstrate the efficacy of our approach.
4. We will use these vectors to transduce wild-type and SOD1 G93A transgenic mice and evaluate the extent of gene targeting, the effect that such silencing has in motoneuron function. This analysis will involve behavioral motor tests, immunohistological analysis and, when needed, electrophysiological recordings in our control and ALS mice. In particular we will test whether these vectors improve the progression of ALS symptoms.

yes	**X**	**Does the project include the possibility of supervised animal manipulation to complete the training for animal manipulator?**
no		