HUMAN MOTOR NEURONS DERIVED FROM INDUCED PLURIPOTENT STEM (IPS) CELLS: A NEW APPROACH FOR MODELING AND TREATING HUMAN SPORADIC ALS.

Delphine Bohl, France
Grant: € 160 000

Amyotrophic lateral sclerosis (ALS) is a severe and incurable neurodegenerative disease. In this disease motor neurons in spinal cord, brainstem and motor cortex are progressively lost and disconnected from their targets. As a consequence patients lose control of voluntary movement and invariably die, most often by respiratory failure. ALS also represents a major socio-economic burden.

Whereas about 10% of all ALS cases are familial and have been associated with highly penetrant mutations, the vast majority of ALS cases is without family history and therefore considered as sporadic. Today, almost nothing is known about the disease mechanisms in sporadic ALS. This is due to the difficulty to obtain human motor neurons and to study them in relevant experimental conditions. Research on sporadic ALS forms is the first priority of this research project. The second aspect of the project relies on the necessity to accelerate development of useful therapeutic strategies for ALS.

To generate human motor neurons, we will use a recently described technique of cellular reprogramming. It consists to genetically reprogram skin fibroblasts into induced pluripotent stem cells, named iPS cells, through the ectopic expression of transcription factors involved in pluripotency.
We have already successfully generated iPS cells from normal human skin fibroblasts (figure A, B).
iPS cells were fully characterized and defined as human pluripotent stem cells. Skin fibroblasts will be obtained from genetic and sporadic ALS patients and age-matched healthy volunteers and iPS cells will be generated and characterized.

Our second objective will be to differentiate iPS cells derived from normal or ALS fibroblasts into motor neurons. We already set up protocols to differentiate iPS cells into neural precursors, neurons (figure C) and into motor neurons expressing the motor neuron marker HB9 (figure D). Protocols will now be improved to increase motor neuron proportions in cultures. To detect motor neurons during the differentiation process, neural precursors will be genetically modified with vectors expressing a fluorescent protein under the control of a motor neuron specific promoter. As different types of motor neurons can be generated from IPS cells and to ensure that iPS-derived motor neurons contain the different motor neuron types potentially affected in ALS, the identity of motor neurons in cultures will be characterized.
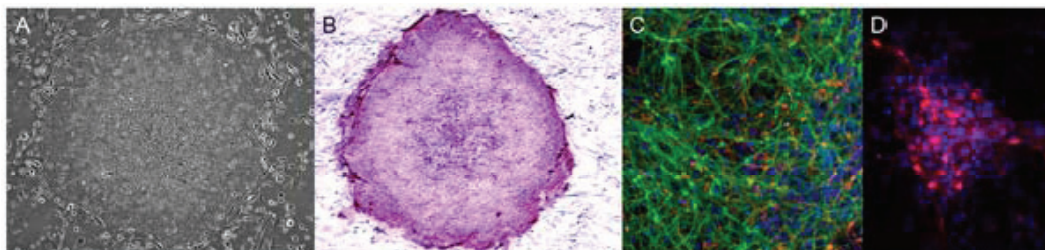
FONDATION
THIERRY LATRAN

Figure. iPS cells characterisation and differentiation. **A**. Phase contrast picture of a human iPS cell clone. **B**. Strong alkaline phosphatase staining is characteristic of iPS cells. **C**. iPS cells can be efficiently differentiated into neurons (in green). **D**. After differentiation, some neurons express the motor neuron marker HB9 (in red).

We will then look whether ALS cells present specific defects. In non-purified cultures containing motor neurons and other cell types including neurons and glial cells, we will analyse cell defects such as the presence of protein aggregates. Cell intrinsic defects in purified motor neurons, including cell survival and neurite length, will be analysed in collaboration with Dr Georg Haase (CNRS, Université de la Méditerranée, Marseille) and within the frame of other projects. If intrinsic defects are detected, we will search whether such defects originate within motor neurons or in their cellular environment.

Finally, the therapeutic potential of normal motor neurons will be assessed in a rat model of ALS. In the last decade, cell therapy has emerged as a new hope for ALS. Despite encouraging results obtained after cell transplantation in animal models of ALS, translation to human patients gave rise to disappointing results, urging the need for more preclinical studies. To accelerate development of useful cell therapy for ALS, we propose to transplant motor neuron precursors derived from normal iPS cells in transgenic mutant SOD1 rats and analyse the therapeutic benefit of this new cell material.

In conclusion, we expect that the generation of iPS cells will allow to model *sporadic* forms of ALS and to better understand the underlying physiopathological mechanisms. We think that human motor neurons genetically reprogrammed from fibroblast's patients might represent a unique extraordinary tool to understand early disease pathways in ALS physiopathology, for development of new biomarkers and, in a more distant future, to develop cell therapies.

TEAM

To reach these objectives our project gathers European groups with complementary expertise in ALS stem cell biology (D. Bohl) and cell transplantation (R. Pochet) benefiting from state-of-the-art facilities of famous Institutes (Institut Pasteur, ULB).

Delphine Bohl and her group (Inserm, Laboratory Rétrovirus et Transfert Génétique, directed by Dr JM Heard, Institut Pasteur, Paris) is specialized in the generation of iPS cells and the directed differentiation of neurons. The team has set up the iPS cell technology using skin fibroblasts from normal individuals. The iPS cell technology was further developed as a contribution to a research program supported by an

ERANET grant for the study of ALS. Here, skin fibroblasts were recently derived from ALS patients with specified genotypes and iPS cells being generated from these cells.

Roland Pochet and his team (Head of the ALS unit within the Laboratory of Histology, Neuroanatomy and Neuropathology of the Faculty of Medicine University of Brussels) is specialized in the electrophysiology and morphology of ALS. They have characterized transgenic rats overexpressing the SOD1G93A mutated protein. Experience in cell transplantation and quantitative follow-up of their location and differentiation is also acquired in the laboratory and recently the surgery of the spinal cord for in situ cell transplantation has been performed successfully on these SOD1 rats.

D. BOHL   S. BLANCHARD   T. LEMONNIER   D. TOLI

R. POCHET   C. NICAISE

ALS-iPSC - Human motor neurons derived from induced pluripotent stem cells (iPSc): a new approach for modeling and treating human sporadic ALS.

Investigators: Delphine BOHL, Retrovirus & Genetic Transfer, Institut Pasteur, France
Participant: Roland POCHET, Université Libre de Bruxelles, Belgique

Updated results December 2012

Human induced pluripotent stem cells, named iPSc, are a unique material to study human neurological diseases. Indeed the iPSc technology is a recent tool allowing the reprogrammation of cells like skin fibroblasts into pluripotent cells having all characteristics of stem cells and having the possibility to be differentiated into any cell type of the human body. This is of particular interest for the study of ALS as the iPSc technology allows for the first time the generation of human motor neurons in a dish and subsequently analyses that could reveal phenotypic differences related to the pathological process.

To model and study ALS, we generated iPSc from collected fibroblasts and iPSc were differentiated into human motor neurons in culture. We observed that in iPSc generated from a patient with a mutant TDP-43 protein, specific TDP-43 protein fragments accumulated in cells a phenomena thought to contribute to ALS pathogenesis. Other analyses focus now on neurite growth defects in ALS motor neurons and the identification of therapeutic coumpounds able to revert such disease phenotypes.

In parallel to these in vivo studies, we performed experiments to evaluate the therapeutic potential of iPSc-derived neural precursors in an ALS rat model with a mutation in the superoxyde dismutase 1 gene (SOD1). Human neural precursors were engrafted in the lumbar spinal cord of pre-symptomatic ALS rats or in control animals. We showed that neural precursors efficiently engrafted and survived into the spinal cord of control and ALS rats for at least two months. A progressive differentiation of neural precursors into mature neurons was observed in rats and neurons with morphologies reminiscent of motor neurons were detected. This work was the first indication that iPSc-derived neural precursors could be engrafted in an ALS environment and were able to survive and generate new neurons. This approach might be relevant to the treatment of ALS associated with SOD1 gene mutation.

Human motoneurons expressing the HB9 specific marker (red) and a neuronal marker (green)