Therapeutic potential of induced pluripotent stem cells in a mouse model of Pompe disease (glycogenosis type II)

Presentation of the Research Unit

Name of the research unit: UMR 703 PAnTher INRA/Oniris, “Animal Pathophysiology and Biotherapy for muscle and nervous system diseases”
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INRA/Oniris UMR 703 is focusing its research activities on the development of therapeutic strategies to combat genetic diseases affecting muscle (Duchenne Muscular Dystrophy) or the central nervous system (motor neuron diseases or lysosomal storage disorders such as metachromatic leukodystrophy as well as glycogenosis type II – Pompe disease). Based on testing the concept with rodents, we have developed our work on gene and cell therapy through the application of large animal models (nonhuman primates, dystrophic dogs, etc.), with the aim of determining the efficacy and safety of the envisaged approaches. The objective of this overall translational research approach is to demonstrate the relevance of the envisaged strategies and implement the preclinical trials required for the development of innovative therapies for human. Our research unit is organized and runs as an integrated multidisciplinary team dedicated to translational research (cell and molecular biologists, confocal microscopist, biochemists, veterinarian surgeon and veterinary pathologists). It includes 24 members: 5 teacher-researchers/researchers, 3 engineers, 7 technicians/assistant-engineers, 2 post-doctoral positions, 4 PhD and 3 master students.

Research Project

Background. The establishment of induced pluripotent stem cells (iPSCs) is an innovative technology that opens up large opportunities in clinic applications and fundamental research. As iPSCs represent an attractive source for obtaining large population of stem cells for regenerative medicine, their medical applications are investigated in several types of disease and stem cell therapy approaches. Pompe disease is a neuromuscular disorder due to a deficiency of acid alpha-glucosidase leading to glycogen storage in many tissues (including heart, diaphragm, skeletal muscles and central nervous system). Pompe disease has been classified into infantile-onset and late-onset forms according to the time of onset and the severity of the disease. Patients with the infantile-onset disease typically manifest hypotonia and signs of heart failure between 3 and 5 months of age. Without treatment, most patients die by the age of 18 months. Currently, the only available treatment is rhGAA enzyme replacement therapy (ERT). ERT has provided an important improvement in survival, respiratory function and cardiomyopathy in patients with the infantile-onset form of disease. However, although symptoms are alleviated, life-long treatment is necessary and the therapeutic effect of rhGAA is variable in skeletal muscles, which remains a significant challenge. Moreover, due to poor uptake of the recombinant enzyme, efficient treatment requires a high dosage that often is associated with an immunogenic response limiting the effectiveness of ERT. Therefore, further efforts are needed to identify novel therapeutic approach for Pompe disease.

Overall aim of the project: launch of a cell therapy approach in the mouse model of Pompe disease to target skeletal muscle and heart using intracardiac and/or intramuscular administrations of murine iPSCs.
**Specific objectives:** using iPSCs tools to rescue expression of acid alpha-glucosidase in mouse model of Pompe disease, we will study:

1) **How the injected murine iPSCs are distributed in heart and skeletal muscle, and what are their biological behavior in targeted tissues**

We have the Pompe disease mouse model (6\textsuperscript{neo}/6\textsuperscript{neo}) of N. Raben, National Institute of Health, Bethesda, USA. This model is phenotypically and genotypically a good model of Pompe disease and the most suitable one for testing cell therapy; both heart and muscular lesions being well characterized (Raben \textit{et al}, 1998). In a first experiment, we will have to demonstrate that intramuscular or intracardiac injections of murine iPSCs previously transduced with nls lacZ retroviral vector can generate the formation of cardiomyocytes and muscle fibers in immunosuppressed 6\textsuperscript{neo}/6\textsuperscript{neo} mice. Four to six weeks later, animals will be sacrificed and tissues will be analyzed by morphological approaches to localize β-gal positive cells.

2) **How the murine iPSCs-mediated restoration of acid alpha glucosidase expression can affect the processing and the storage of glycogen and lead to heart and skeletal muscle phenotype correction**

We will demonstrate which therapeutic strategy with murine iPSCs could be safe and efficient to achieve a heart and muscle phenotypic recovery in Pompe disease. We will test intramuscular and intracardiac delivery in adult mouse model for Pompe disease to assess the phenotypic correction in heart and skeletal muscle. Animals will be followed at mid term, monitored by functional tests (in collaboration with Corinne Cadiou, UMR_S 915, Thorax Institute, Nantes) then compared to mock-injected Pompe disease affected mice to look for any phenotypic correction. A biochemical approach will be performed using western blot analysis, enzyme activity measurements and glycogen assays on heart and muscle samples, in combination with assessment of phenotypic correction (by histology, immunohistochemistry and confocal microscopy) to prove the efficiency of our strategy.

**Research Environment**

These rely on cell and gene, and animal models (Pompe disease). We modify cell content and analyze the clinical, histological and biochemical phenotypes of the different models as well as the immune response. Analysis of animal phenotype requires a combination of behavioural studies, \textit{in vivo} studies of gene and protein expression, biochemical and neuropathological analysis (“mouse and large animal medicine”) and is performed in the laboratory and in the Gene and Cell Therapy Center (Oniris). The majority of the heavy equipment is available in the research unit and at the Oniris site. A certified BL2 laboratory was built in 2009 for rodents at the Oniris animal facility to house of viral vector treated transgenic animals. Certified BL2/BL3 laboratories are allocated at the Gene and Cell Therapy Center for the housing of GRMD dogs and nonhuman primates.

**List of most recent publications of the laboratory:**