Modulation of autophagy and glucose metabolism in 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”

Address: Oniris, Nantes-Atlantic College of Veterinary Medicine and Food Sciences

Location: Nantes, France

Laboratory Director: Professor Marie-Anne COLLE, DVM, DiplECVP

Scientific supervisor for the project: Dr Carine CIRON

Phone number: 33 2 40 68 28 07

Email address: carine.ciron@oniris-nantes.fr

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. Pompe disease, a metabolic myopathy with heterogeneous clinical presentations, is caused by lysosomal acid alpha-glucosidase (GAA) deficiency leading to progressive accumulation of glycogen in heart, muscles and central nervous system (CNS). The clinical spectrum ranges from fatal hypertrophic cardiomyopathy and skeletal muscle myopathy in infants to relatively attenuated forms, which manifest as a progressive myopathy without cardiac involvement. The currently available therapy, replacement of the missing enzyme by intravenous infusion (ERT), proved to be successful in correcting cardiac abnormalities but failed to reverse skeletal muscle. The etiology of this pathology is more complex than just a lack in GAA.

Overall aim of the project In the years to come, we will put a lot of efforts into a better understanding of the pathogenesis in Pompe disease, particularly in muscles. Lysosomal enlargement/rupture as long been proposed to account for skeletal muscle destruction. However, it became clear that this simple view of the pathology is inadequate; the pathological cascade involves dysfunctional autophagy, a major lysosome-dependent intracellular degradative pathway. In muscles of Pompe patients, large areas with autophagic build-up were found.

Specific aims:

Over the next few years, we will study:

1) How autophagy could be detrimental or not in the pathogenesis of Pompe Disease
2) How we can act on autophagic flux or endocytic pathway to alleviate the muscular pathology and promote the accessibility of the recombinant enzyme into lysosomes.
1) How autophagy could be detrimental or not in the pathogenesis of Pompe Disease

The Pompe mouse model, 6neo/6neo, a colony actually housed in our animal facility, displays many feature of the human disease an in particular massive autophagic build-up are found in skeletal muscle cells. They also showed enlarged lysosome with excessive glycogen storage. In order to study autophagy in whole animals, mice expressing green fluorescent protein (GFP) fused to autophagosomal marker LC3 have been generated. This is a unique model to monitor the formation of autophagosomes in vivo and per se autophagy flux. We will cross our 6neo/6neo mice with LC3-GFP mice in order to obtain double transgenic mice: LC3-GFP/Pompe. The GFP tag will allow us to easily and accurately identify autophagic structures in living animals using our two-photon imaging. These double transgenic mice will constitute an innovative tool to monitor autophagy in vivo. To understand how the autophagic flux is impaired in Pompe Disease, we will challenge these mice with drugs promoting or decreasing autophagy and we will follow the formation of autophagosomes in vivo. We will also collect different tissue samples at different time points to study the autophagic pathway with a molecular and biochemical perspectives.

2) How we can act on autophagic flux or endocytic pathway to alleviate the muscular pathology and promote the accessibility of the recombinant enzyme into lysosomes.

The Forkhead transcription factor, FoxO3, at the interplay between glucose metabolism and autophagy, appears as a highlighting therapeutic target. The FoxO family members constitute an important metabolic sensor by promoting gluconeogenesis and glycogenolysis. FoxO3a regulates a host of metabolic enzyme for glucose metabolism. Moreover, FoxO3 plays a critical role in muscle atrophy and is necessary and sufficient for the induction of autophagy in skeletal muscle in vivo. The study of FoxO3 pathway may offer new therapeutical perspectives by acting both on autophagy and glucose metabolism. We will determine how the modulation of FoxO3 activity by interacting with autophagy pathway and glucose metabolism can be beneficial in our LC3-GFP/Pompe model in vivo. We will use mutants and wild-type forms of FoxO3 encoding in AAV vectors. All vectors will be produced by the vector core facility of INSERM UMR 1089. We will monitor autophagy in response to each form of FoxO3a in skeletal muscles throughout the study. Moreover, glucose and insulin tolerance test will be achieved at different time point. At euthanasia, tissue samples will be collected to evaluate autophagy and glycogen storage in skeletal muscles. Western blot analysis, GAA activity and glycogen content will be performed in collaboration with C. Caillaud (biochemist, Cochin Institute). Assessment of phenotypic correction will be based on the strong experience acquired in muscle by the unit.

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, 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 environment is performed in the laboratory and at the Oniris site. A certified BL2

List of most recent publications of the laboratory: