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**Differential gene and miRNA profiling in dystrophic dog and impact of MuStem cell-based therapy**

**Florence Rodrigue**1,2,3,4, Aurélie Lardenois1,2,3, Candice Babarit1,2,3, Thibaut Larcher1,2,3, Mireille Ledevin1,2,3, Laurence Dubreil1,2,3, Chantal Thorin2, Laetitia Guevel1,2,3,4 and Karl Rouger1,2,3

1INRA UR1753, F3423 Vet, F-44307 Nantes. 2LUMINUM Université, Nantes, Ecole nationale vétérinaire, agro alimentaire et de l'environnement Nantes-Atlantique, F-44307 Nantes. 3Atlantic Gene Therapy, F-44000 Nantes, France. 4Université de Nantes, F-44322 Nantes, France. 5Laboratoire de Physiopathologie Animale et Pharmacologie fonctionnelle, F-44307 Nantes, France.

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**Introduction** - Duchenne Muscular Dystrophy (DMD) is a progressive fatal X-linked recessive disorder of skeletal and cardiac muscles that affects 1 in 3,500 male births. It is characterized by the lack of dystrophin protein at the sarcolemma that results in chronic degeneration/regeneration of muscle fibers and is clinically associated with a progressive muscle weakness. Currently, there is no therapy to cure DMD. Over the last few years, the Golden Retriever Muscular Dystrophy (GRMD) dog model has been increasingly used to assess efficacy of a wide range of gene- and cell-based therapy approaches. Numerous studies are ongoing to define muscle molecular signature that could be used to characterize the dystrophic dog tissue and/or to validate the effect of therapeutic strategies. Recently, we have shown that systemic delivery of MuStem cells, muscle-resident stem cells isolated from healthy dog based on delayed adhesion properties, represents an attractive avenue for future therapeutic applications in DMD patients. Indeed, allogenous MuStem cell transplantation generates the remodeling of muscle tissue and gives rise to striking clinical benefit in GRMD dog (Rouger et al., 2011). This global effect, linked to a relative low dystrophin protein level, leads us to question here the molecular pathways that are impacted by MuStem cell transplantation.

**Objective** - The aim of the study was to establish a high-throughput characterization of GRMD dog muscle and also to determine the molecular consequences of MuStem cell delivery.

**Experimental context:** Systemic delivery of MuStem cells

**Material and methods**

We performed comparative transcriptomic and miRNA studies between healthy, mock GRMD and transplanted GRMD-dog muscles in order to identify changes in transcript and miRNA profiles.

- **Transcripomic analysis:**
  - **Biceps femoris** muscle samples were collected surgically from the middle portion of the muscle in 3-month-old healthy, mock GRMD dogs and GRMD-dog muscles (n=2). Total RNA was extracted from 50 mg of frozen muscle and quantified. Each sample was made by pooling two independent extractions of the same muscle and was treated with DNase.
  - The transcriptomic experiment was performed by the integrative Genomic Platform (Nantes, France). miRNA expression profiling was obtained using the Agilent 021430 Canine (V2) Gene Expression Microarray.
  - Statistical filtration and classification were performed. The detectable probes yielding signals ≥ 7.32 were identified and probes displaying a high expression variation (fold change ≥ 2) were then selected. ALRMAP statistical test was done.
  - **miRNA study:** Twelve 3-month-old golden retrievers were included in the study: 3 healthy and 9 GRMD dogs. GRMD dogs were divided in three groups: 3 dogs received neither immunosuppressive regimen nor cell transplantation; 3 others received continuous immunosuppressive regimen (named mock GRMD) and the last three ones received MuStem cell transplantation under immunosuppression (named GRMD-MuStem).
  - The expression levels of 6 miRNAs (miR-23, miR-123, miR-206, miR-34 and miR-486) were determined by RT-qPCR in Biceps femoris of all dogs. In order to analyze simultaneously the expression level of all miRNAs in the different dogs, a Principal Component Analysis (PCA) was performed. A Pearson correlation test was applied to the expression of each miRNA with the two first axes of PCA. A confidence ellipse was drown for each dog subset considering a 95% confidence level.
  - The cellular localization of miR-206 and miR-486 was assayed by in situ hybridization on cryostat muscle sections.

**Transcriptomic study**

**A:** Profiling of differentially expressed transcripts

**B:** Functional characterization of GRMD dog model

**C:** Impact of MuStem cell delivery

**miRNA study**

**A:** Differential miRNA expression in GRMD dog muscle

**B:** miR-206 and miR-486 localization in GRMD dog myofibers

**Conclusions**

In order to characterize the global perturbations of the dystrophic canine model and to identify potential biomarkers of the cell therapy efficiency transcriptomic and miRNA studies were performed. Interestingly, we show that MuStem cell administration is associated with expression pattern modification of many factors belonging to different biological functions such as muscle mass regeneration, metabolism, lipid and energy homeostasis. In addition, we demonstrate a corrective effect of MuStem cell transplantation on the muscle expression of miR-133 and miR-486.

The combination of these approaches allows to precisely the pathophysiological events that trigger the muscle fiber degeneration in DMD model and paves the way to the understanding of MuStem cell action modalities.

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