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Exploration of muscle from GRMD dogs transplanted with MuStem cells using « omics » approaches

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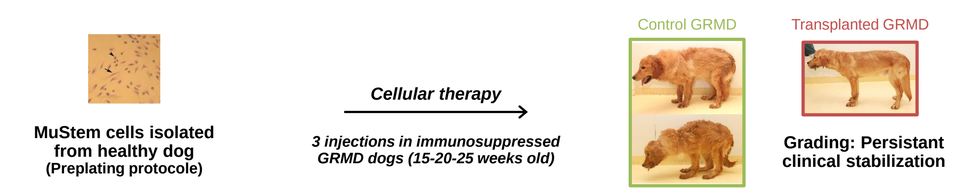
Introduction -

Duchenne Muscular Dystrophy (DMD) is a fatal X-linked recessive muscle disorder that affects 1 in 3,500 male births. It is characterized by a lack of dystrophin protein at the sarcolemma of muscle fibers 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. During the last decade, one suggested route to surmount the limitations of myoblast transplantation proposes the use of **adult stem cells** that have been demonstrated to exhibit myogenic potential in experimental conditions.

Among the Muscle-derived Stem Cells (MDSCs), our lab recently isolated from skeletal muscles of healthy dog a marginal fraction of delayed adherent cells named canine **MuStem** cells (cMuStem) and demonstrated that their systemic delivery in clinically relevant Golden Retriever Muscular Dystrophy (GRMD) dogs model results in muscle damage limitation and persisting stabilization of the dog's clinical status (Figure 1). These data provide evidence that allogenic MuStem cell transplantation could represent an attractive tool for therapeutic application in DMD context (Rouger *et al.*, 2011).

To pave the way to the understanding of the **mechanisms underlying the therapeutical effect of the cMuStem cells** and identify **potential biomarkers of the treatment efficiency** we employed combined "omics" approaches that allowed us to identify changes in transcript, miRNA and protein profiles in *Healthy*, *GRMD* and *Treated GRMD* muscles.

Systemic delivery of cMuStem cells (Rouger *et al.*, 2011)



▲ **Figure 1 – MuStem cell, a candidate for DMD cellular therapy.**

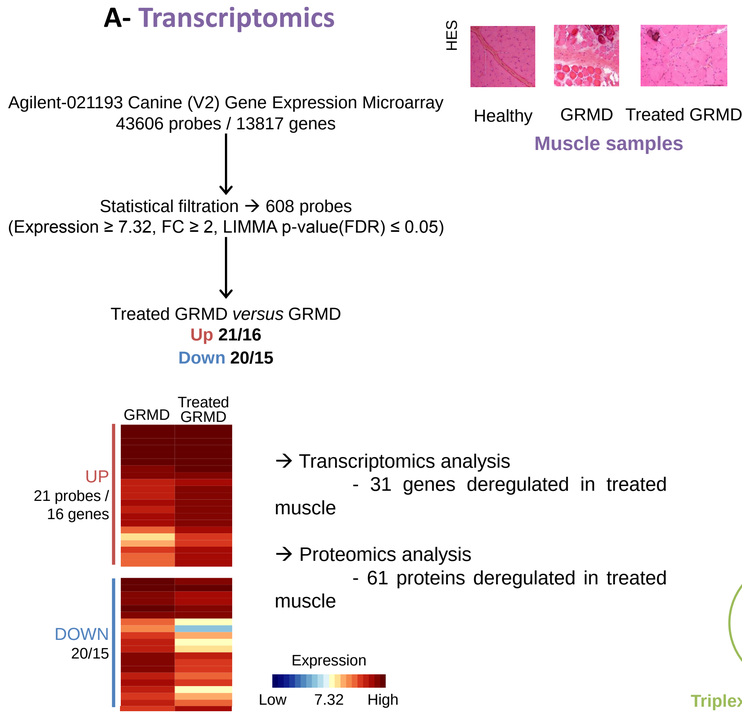
Combined "omics" strategy

To focus this study on the treatment effect, the treated GRMD muscle samples were compared to GRMD muscles.

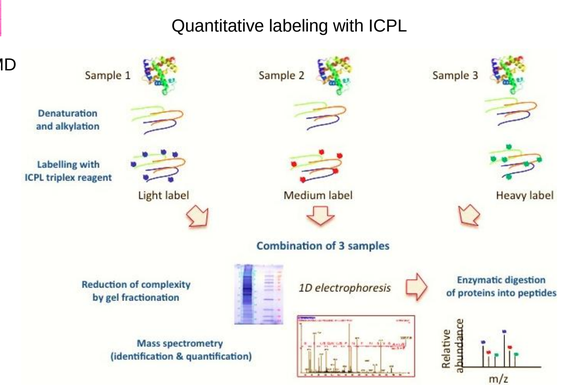
Transcriptomics microarray data from canine Agilent array and quantitative proteomics data from labeling with ICPL (Isotope-coded protein labeling) were separately analyzed (Figure 2, Panels A and B). In parallel, miRNA exploration in muscle and serum are also under process.

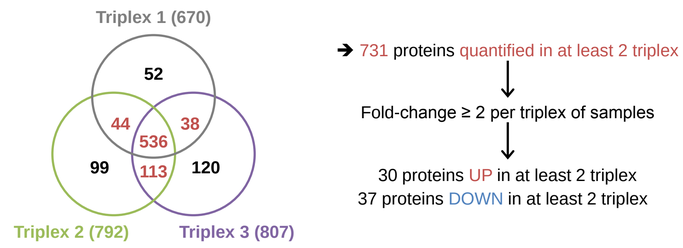
Both transcriptomics and proteomics data were further combined in order to better understand the treatment effect (Figure 3, Panel A).

A- Transcriptomics



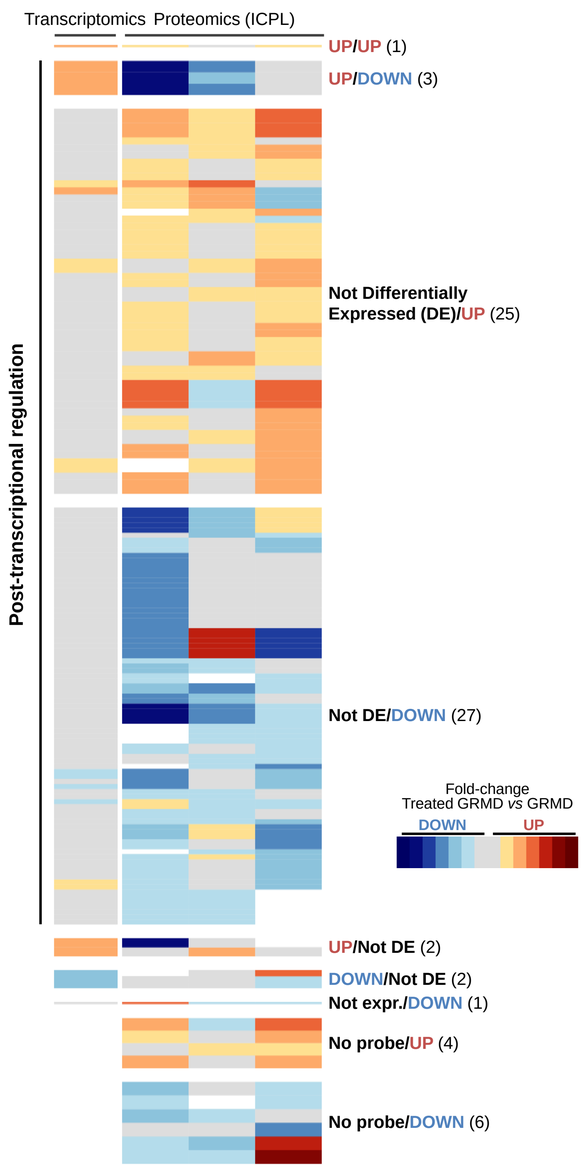
B- Proteomics





▲ **Figure 2 – "Omics" strategy for the exploration of the cMuStem cell therapy.**

A- Data integration

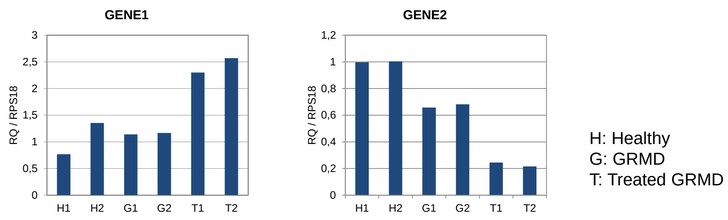


B- Functional analysis of integrated data

Gene ontology		Not DE/UP	Not DE/DOWN
biological_process (7 terms)	29993	23	23
0006916 anti-apoptosis	628	**5 / 1**	1 / 1
0008064 regulation of actin polymerization or depolymerization	292	**4 / 0**	0 / 0
0030049 muscle filament sliding	121	1 / 0	**4 / 0**
0006936 muscle contraction	555	2 / 0	**7 / 0**
0007517 muscle organ development	1011	2 / 1	**7 / 1**
0006937 regulation of muscle contraction	285	0 / 0	**5 / 0**
0008015 blood circulation	767	1 / 1	**5 / 1**
molecular_function (3 terms)	27212	20	22
0005509 calcium ion binding	1609	**6 / 1**	**7 / 1**
0008307 structural constituent of muscle	129	**2 / 0**	**6 / 0**
0005200 structural constituent of cytoskeleton	243	1 / 0	**3 / 0**

Depleted — p-value — Enriched
1 — 0.95 — 0.05 — 1e-5 — 0

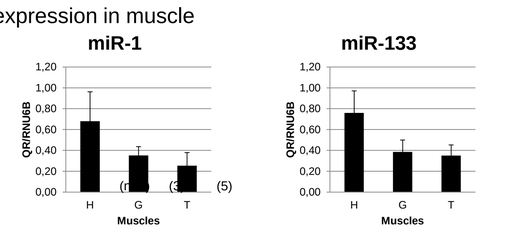
C- Experimental validation of transcriptomics data by RT-qPCR



→ Validation of 21 targets differentially expressed

D- Post-transcriptional regulation ⇨ miRNA study

- miRNA expression in muscle



→ No significative changes of myomiRs in treated GRMD muscle
→ Work in progress :
- to identify new miRNA deregulated in muscle
- to find potential miRNAs as biomarkers in serum

▲ **Figure 3 – Proteomic results integrated with the transcriptomic data**

Results – Treated GRMD *versus* GRMD

Functional analysis We found that the set of over-expressed proteins was composed of factors involved in anti-apoptosis through an anti-inflammatory activity, in actin cytoskeleton reorganization and in calcium signaling (Figure 3, Panel B).

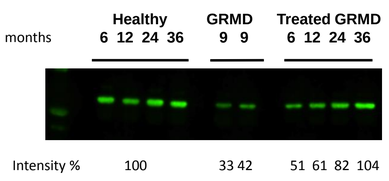
Here, the MuStem cell therapy has counter-acted protein alterations in cytoskeletal microtubules components such as myosin light chains. Other muscle structure and regeneration proteins, over-expressed in GRMD muscle (Guével *et al.*, 2011) reveals reversal level in treated dog.

The combination of these data identified a single protein over-expressed that was also up-regulated in transcriptomics (Figure 3, Panel A top). This proteins is known to play a role in muscular regeneration.

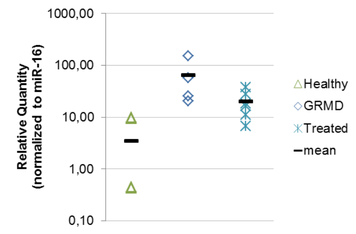
The vast majority of the proteins differentially expressed between treated GRMD and GRMD samples was not differentially expressed at the transcript level, meaning that their expression seems to be under the control of post-transcriptional regulation (Figure 3, Panel A). Consequently, we will focus on miRNA study to validate this hypothesis.

Potential biomarker discovery

- **Secreted protein in serum**



- **miRNA expression in serum**



→ The potential protein biomarker identified by the transcriptomics analysis is down expressed in GRMD while it increases in treated GRMD with age to exhibit a level similar than those shown in healthy dog.
→ At 9 months, the expression of the identified miRNA decreases in treated GRMD samples to get closer to the healthy dog muscle samples.

Conclusion and discussion

In order to characterize the global perturbations of the dystrophic dog model and to identify potential biomarkers related to the cell therapy treatment efficiency we performed an integrative « omics » strategy.

Here, our study showed how transcriptomic and proteomic profiling could be used for the proper evaluation of novel therapeutical approaches. Our strategy for MuStem cell therapy evaluation in GRMD dog analysed the reversal abnormalities in treated muscles with non-dedicated approaches.

By jointly using genomics and proteomics there is a great potential to make considerable contribution to potential biomarkers identification and to open up new avenues for characterization of the physiopathological events, and evaluation of new therapies.

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