Exploring the protective role of GDF5 against skeletal muscle disuse atrophy

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Goodmorning to everyone my name is Chiara Noviello and I am a post doc in France Pietri Rouxel team an the Institut of Myology in Paris and today I will present preliminary data on the role played by Gdf5 in muscle atrophy caused by disuse

Everybody knows that skeletal muscle is a high plastic tissue cause it is able to change its mass depending on external stimuli. For instance following excercise it hypertrophies or on the contrary aging, denervation, starvation, pathological condition or immobilization lead to muscle atrophy

In particular, in this talk I will focus on immobilization that is a process related to several factors like unbalance between protein synthesis and protein degradation, increased oxidative stress, increased inflammation, switch of fiber type, decreased force and deregulated oneuro muscular junction and cross talk muscle nerve.

Many researches in the lab are focused on Gdf5, the growth differentiation factor. It belongs to BMP family and its role in bone and cartilage formation is well described, however its role in skeletal muscle is poorly understood. It is a secreted protein and once that it binds BMPreceptor it activate smad 1/5/8 phosphorilation. Once phosphorylated recrute smad4 and togheter translocate into the nucleus to regulate their target genes expression.

A recent publication in the lab showed that the axis gdf5/cavb1, important to regulate muscle mass homeostasis, positively regulates muscle mass; in particular when CaVbeta1E or GDF5 are overexpressed in TA muscle in denervated condition in aged mice, muscle mass is preserved.

Moreover recent data of our lab not yet published showed that the supplementation of ricombinant gdf5 in aged mice is able to increases muscle mass, improves muscle force and ameliorates neuromuscular connectivity.

So we wonder if gdf5 could play a role in muscle atrophy due to immobilization and so if it could be considered as a therapeutic approach to counteract muscle atrophy in disuse condition

In order to answer to this question we injected in intramuscular way young mice with an AAV in order to overexpress or not gdf5 and 10 days later we immobilized one hindlimb of the mouse with an hook and loop fastener method. This is a less invasive approach that allow an efficient atrophy of the hindlimb muscles. The eppendorf tube and the staple keep the knee and ankle plantar in extension position avoiding any joint mouvement.

At first we verify the overexpression of Gdf5 after AAV injection. as you can see in the graph gdf5 is well overexpressed in ta, whether in edl and gas that are near to injection site gdf5 overexpression in very low.

Later we verified the atrophy induced by immobilization and we observed that we obtain a significant decrease of all muscle mass of the hindlimb with an atrophy from 15 to 22 % compared to contralater leg

when we overexpress Gdf5 we do not have any effect of Gastrocneloius muscle. However Edl muscle mass is partially rescued by gdf5 overexpression after immobilization. Surprinsgly Ta muscle mass has a very slight not significant increase meaning that the effect of Gdf5 overexpression is muscle type specific

To go further we analysed the hindlimb muscle by mri imaging and we observed that after Gdf5 overexpression the inflammatory infiltrate is reduced as you can see clearly from the images. Moreover when we analysed the gene expression of the one of the most important pro-inflammatory cytokines Il1B we observed that after immobilization its expression significantly increase but after gdf5 overexpression is mantained at basal level suggesting that gdf5 riduces the inflammation state occurring during immobilization

As I mentioned in the introduction immobilization induces a deregulation of nmj and a rapid neuromusuclar remodeling. Indeed after immobilization there is an increase of the expression of genes encoding for the subunits alfa, delta and gamma of acetilcolin receptor. This increase attempt to overcome the imposed disuse and mechanical resistence in a sort compesatory mechanism

Interestingly after gdf5 overexpression this increase is even more as showed by rt-qpcr results. These data suggest that Gdf5 amplify the hypersentivity of nmj stimulating and improving its responsiveness to the external stimulus of disuse

Going further we analyzed nmj morphology on single isolated myofibers and we showed that endplate area that usually decreases upon muscle disuse is maintained at basal level by gdf5 overexpression and that the overlap between achr and neurofilament is significantly better after gdf5 overexpression, meaning that gdf5 improves nmj functions and ameliorate muscle innervation in disuse condition. These data are corroborated by gene analysis that showed a significant increase of Gap43 expression that is a gene responsible for nerve sprouting and axon regeneration.

In conclusion we can assert that different muscles respond in a different way to gdf5 overexpression Reduce inflammation in disuse condition and ameliorate nueromuscular connectivity

In the perspectives we want to undestand why gdf5 has not the same effect on diferent muscles analysing BMPR expression for instance in order to design a more specific treatment
Indeed the next step is to develop a more therapeutic approach for example using a recombinant gdf5 in sistemic way to counteract muscle waste during immobilization period
We would like to investigate the molecular mechanisms underling gdf5 effects using in vitro approach where muscle cell line are cultured in absence of gravity.