"Circulating myomiRs as markers of myogenic precursor abundance"

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Project overview

Skeletal muscle size is dynamic and responsive several stimuli, among which exercise. However, while resistance exercise results in hypertrophy and increased force generation, endurance exercise leads to increased expression of the peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC-1α) and increased number of mitochondria, with little changes in the skeletal muscle mass. Both types of exercise stimulate the proliferation of satellite cells, muscle-specific stem cells that will subsequently differentiate and fuse with existing myofibers. Finally, exercise also increases circulating microRNAs (miRs), short noncoding RNAs that post-transcriptionally modulate gene expression. Some miRs (myomiRs: miR-1, miR-133a, miR-133b, miR-206) are abundant in the skeletal muscle and appear modulated in muscle atrophy, hypertrophy and myogenesis.

Autologous transplantation of myogenic stem cells has been proposed as the next generation doping strategy. The number of myogenic precursors may be increased both 'artificially' by autologous implantation, or physiologically, by regular exercise. However, while all myogenic precursors are increased by the implantation, exercise mainly leads to satellite cell accumulation.

The target of the study is to define if autologous myogenic stem cell implantation may effectively impinge on muscle mass and function and to clarify if circulating myomiR levels can be useful as biomarkers of myogenic stem cell abundance and type. The study will be developed by using transgenic mice hyperexpressing PGC-1α specifically in the skeletal muscle. These mice mimic the condition of endurance-trained subjects, being characterized by high exercise performance. Briefly, circulating myomiRs will be evaluated in these mice, and correlated with the abundance and type of myogenic stem cells. Subsequently, myogenic stem cells isolated from wild-type and transgenic animals will be implanted into wild-type recipients, to test the effectiveness of the procedure in improving muscle mass/function. The results obtained with the transgenic mice will be validated on human volunteers.

Results and Conclusions:

Skeletal muscle size is dynamic and responsive to several stimuli, among which exercise. However, while resistance exercise results in hypertrophy and increased force generation, endurance exercise leads to increased expression of the peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α (PGC-1α) and increased number of mitochondria, with little changes in the skeletal muscle mass. Both types of exercise stimulate the proliferation of satellite cells, muscle-specific stem cells, that will

subsequently differentiate and fuse with existing myofibers. Finally, exercise also increases circulating microRNAs (miRs), short noncoding RNAs that post-transcriptionally modulate gene expression. Some miRs are modulated in muscle atrophy, hypertrophy and myogenesis.

Autologous transplantation of myogenic stem cells has been proposed as the next generation doping strategy. The number of myogenic precursors may be increased both 'artificially', by autologous implantation, or physiologically, by regular exercise. The target of the study has been to define if: 1)autologous myogenic stem cell implantation may effectively impinge on muscle mass and function; 2) circulating miR levels can be useful as biomarkers of myogenic stem cell abundance and type. The study has been developed by using transgenic mice hyperexpressing PGC-1α specifically in the skeletal muscle (MCK-PGC-1α). These mice are characterized by a high amount of myogenic stem cells compared to wild-type (WT) animals, and mimick the condition of endurance-trained subjects, being characterized by high exercise capacity.

The effects of the infusion of myogenic precursors isolated from WT or MCK-PGC-1α mice into Tibialis anterior (TA) muscles of WT hosts, either undergoing muscle damage or not, have been evaluated. Transplantation of myogenic precursors isolated from WT animals does not alter TA morphology and metabolism. Surprisingly, WT cell injection after muscle damage is able to restore the physiological muscle phenotype. Interestingly, when myogenic cells deriving from MCK-PGC-1α animals are infused, a shift towards the oxidative phenotype in both damaged and undamaged muscles can be observed. Histological analyses put in evidence small and oxidative fibers, while molecular markers show an increase in mRNA or protein expression levels of many markers related to myogenesis (Pax-7, Myogenin, embryonal Myosin) and mitochondria (PGC-1α, Tom20). These results suggest that the engraftment of WT myogenic precursors improve muscle regeneration after severe injury. In addition, PGC-1α overexpression might favor myogenic differentiation and affect regeneration by converting the canonical mixed phenotype of a muscle into the oxidative one, endowed with increased exercise capacity. These findings, potentially relevant to regenerative medicine aimed to improve several diseases, may also open the way to a non-ethical use as doping strategy.

As for the second aim of the study, circulating miR profile is comparable between WT and MCK-PGC-1α mice. Such result could be explained by the observation that MCK-PGC-1α mice in resting condition do not display a specific phenotype: the enhanced capacity of the skeletal muscle system, indeed, becomes evident upon stimulation, for example by exercise. Indeed, circulating levels of miR-21-5p and miR-181-5p are increased in exercised WT animals while not in the MCK-PGC-1α mice allowing to hypothesize that stress conditions up-regulate these miRs in WT animals, while the oxidative phenotype and the abundance of myogenic precursors occurring in MCK-PGC-1α mice likely improve the management of stressing stimuli. These results have been compared with those obtained in human volunteers practicing combined exercise for 3 months. Despite the small number of subjects

included in the study, a trend towards increase can be envisaged for miR-27-3p, miR-133a-3p and miR-181-5p. This latter, in particular, follows the same trend in both exercised WT mice and volunteers. Despite the lack of robustness, these data suggest that circulating levels of selected miRs can be up-regulated by exercise, and that such modulation can be prevented by genetic manipulations able to improve myogenic precursor abundance and muscle oxidative phenotype. In particular, it could be proposed that low circulating levels of miR-181-5p in athletes suggest a shift of muscle metabolism from glycolitic to oxidative; this might reflect the infusion of autologous stem cells, either untreated or genetically modified. In this regard, the detection of low miR-181-5p levels in the circulation could be useful for a first screening, identifying subjects deserving more accurate analysis.

Publications / presentations related to the project

1. PGC-1α overexpression in the skeletal muscle: effects on myogenesis
Marc Beltrà, Fabrizio Pin, Riccardo Ballarò, Ambra Iannuzzi, Fabio Penna, Paola Costelli. 14th Interuniversity Institute of Myology Meetings, October 12-15, 2017, Assisi (PG), Italy. *Oral presentation*

2. PGC-1α overexpression in the skeletal muscle: effects on myogenesis
Marc Beltrà, Fabrizio Pin, Riccardo Ballarò, Ambra Iannuzzi, Fabio Penna, Paola Costelli. 10th International Conference on Cachexia, Sarcopenia and Muscle, December 8-10, Roma, Italy. *Poster presentation*