Regulating muscle protein synthesis in human myotubes from young and old subjects with microRNAs

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Background and Aim: Sarcopenia is the age-related loss of muscle mass and function. The onset and progression of sarcopenia is associated with attenuated activation of Akt/mTOR signalling and muscle protein synthesis (MPS) in response to anabolic stimuli such as resistance exercise. MicroRNAs (miRNAs) are short strands of nucleic acids that can influence muscle growth. The regulation of Akt/mTOR signalling and MPS by skeletal muscle miRNAs is poorly understood. A miRNA screening followed by statistical and bioinformatics analysis allowed the identification of 2 miRNAs that potentially regulate MPS in young and old human subjects. MiR-99b and miR-499a are both differentially regulated by age and resistance exercise and directly target, or are predicted to target, members of the Akt/mTOR signalling pathway. This study aims to validate the role of miR-99b and miR-499a as putative negative regulators of MPS *via* Akt/mTOR signalling in human primary myotubes.

Methods: Primary myocytes were isolated from the biopsies of three young (23.5±5.0 y.o.) and three old (64.6±4.0 y.o.) subjects. Myotubes were transfected with mimic sequences to increase the levels of miR-99b or miR-499a. The control groups were treated with a nonsense mimic sequence. *In vitro* protein synthesis was directly assessed by the incorporation of ^{3}H-tyrosine into the myotubes. The expression levels of predicted miRNA targets known to regulate muscle protein synthesis will be determined by qPCR and western blot. Differences will be determined by 2-way ANOVA.

Results: *In vitro* protein synthesis levels have been determined for 5 of the 6 cell lines. Overall, basal protein synthesis levels were lower in the old cell lines when compared to the young cell lines ($P<0.05$). The miR-99b mimic significantly decreased protein synthesis levels in both young and old cell lines ($P<0.001$). We observed a significant age by treatment effect for the miR-499a mimic ($P<0.001$) with a significant reduction in protein synthesis in the old but not young cell lines treated with the miR-499a mimic.

Conclusions: This study provides the first proof of concept for the role of miRNAs as regulators of protein synthesis in human myotubes. MiR-99b and miR-499a mimics both negatively regulate protein synthesis in young and old myotubes. To elucidate the mechanism of action of these miRNAs, the expression levels of validated and predicted miR-99b and miR-499a targets will be measured as well as the phosphorylation level of regulators of the Akt/mTOR pathway.