MiR-499 reduces muscle protein synthesis in human primary myotubes

E. Zacharewicz, P. Della Gatta, L. Ghobrial, A.P. Russell and S. Lamon, Centre for Physical Activity and Nutrition (C-PAN) Research, School of Exercise and Nutrition Sciences, Deakin University, Burwood, VIC 3125, Australia.

Introduction: Progressive loss of skeletal muscle is a natural phenomenon that occurs as we age, resulting in frailty and reduced mobility. The loss of skeletal muscle mass is associated with an attenuation of muscle protein synthesis (MPS) following an anabolic stimuli. However, the underlying molecular mechanisms responsible for the reduction in MPS are not well understood. Our group identified miR-499, a microRNA (miRNA) predicted to regulate MPS *via* Akt-mTOR signalling, to be dysregulated in the skeletal muscle of old subjects. MiR-499 is predicted to regulate eIF4G2, an eukaryotic initiation factor essential for protein translation. The aim of this study was to validate the role of miR-499 as a negative regulator of MPS in human primary myotubes derived from young and old subjects.

Methods: Myocytes were isolated from the biopsies of 3 young (23.5±5.0 y.o.) and 3 old (64.6±4.0 y.o.) subjects. Myotubes were transfected with a miR-499 mimic sequence for 8 h to increase the intracellular levels of miR-499. Cells were harvested 24 h after the onset of mimic transfection. Protein synthesis was assessed by the incorporation of 3H-tyrosine into the myotubes. The gene and protein levels of eIF4G2 and members of the Akt-mTOR signalling pathway were assessed by qPCR and western blot. Reporter assay was used to determine if there was a binding interaction between miR-499 mimic and its predicted target sequence within the eIF4G2 3′ UTR. Differences between groups were determined by a 2-way ANOVA.

Results: Overexpression of miR-499 significantly reduced protein synthesis over a 16 h period following transfection in human primary myotubes derived from old subjects only ($P<0.001$). eIF4G2 gene expression was reduced with miR-499 overexpression ($P<0.001$). However, no binding interaction between the miR-499 mimic and its predicted target within the eIF4G2 3′ UTR was observed with the reporter assay. There was also no difference in eIF4G2 expression at the protein level. No changes were observed in the phosphorylation levels of Akt-mTOR signalling proteins with the exception of 4EBP1, which was increased in the myotubes derived from old subjects following transfection.

Conclusion: Overexpression of miR-499 in human primary myotubes reduces protein synthesis in cell lines derived from old subjects only. This reduction in protein synthesis was associated with an increase in the phosphorylation levels of 4EBP1 protein. A reduction in eIF4G2 gene expression, a predicted target of miR-499, was also observed; however a direct binding interaction between miR-499 and eIF4G2 could not be established. Further work will be carried out to elucidate how miR-499 regulates protein synthesis and where the age-specific effect in the protein synthesis response occurs with miR-499 overexpression.