Al Collegio Docenti del Dottorato in Medicina Molecolare

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Introduction

Rs508419: C>T is a SNP located in the muscle-specific ANK1 gene internal promoter, driving the transcription of the sANK1.5 isoform in striated muscle. Through GWAS, it was shown that the C/C allelic variant is associated with type 2 diabetes (T2D) susceptibility in humans. This variant is responsible for a higher activity of the internal promoter resulting in increasing levels of sAnk1.5 mRNA and protein. Interestingly, C2C12 cells over-expressing sAnk1.5 show a reduction in glucose uptake. MiR486 is located in intron 40 of the ANK1 gene and, is transcribe starting from by the same internal promoter as sANK1.5. Since miR-486 has been shown to target several members of the insulin-like growth factor (IGF) signalling pathway, it may be expected that it's expression also correlates with type 2 diabetes susceptibility.

Methods

Genomic DNA was extracted from 260 human muscle tissue biopsies collected between 2004 and 2019. Primers specific for amplification and sequencing of the ANK1 region containing the SNP were designed. To evaluate sANK1.5 and miR486 expression levels, total RNA and microRNAs were extracted from the same bioptic samples used for the genetic screening. Total RNA and microRNA were retro-transcribed and the cDNA was analysed by qPCR. The relative expression level was assessed following amplification of the β-actin housekeeping gene for sAnk1.5 and of the U6 RNA for miR486.

Results

On a total amount of 260 human samples, we obtained a distribution of 137 subjects carrying the C/C allelic variant, 105 the C/T and 18 the T/T. This distribution is in accordance with the allele frequency reported by the HapMap database. We selected 18 C/C, 18 C/T and 18 T/T to be tested for the evaluation of sAnk1.5 mRNA and miR486 expression levels. Samples carrying the allelic variant C/C showed higher levels of both sAnk1.5 mRNA and miR486 when compared to the sample carrying the T/T allelic variant, thus suggesting a common regulation of sAnk1.5 and miR486 expression. These results confirm that the C/C variant causes a significant increase in the activity of the internal muscle-specific promoter of ANK1 gene, resulting in higher levels of sAnk1.5 mRNA and miR486. The following steps of this study will be aimed to investigate the effect of increased levels of miR486 on the expression of its downstream targets and on the molecular pathways regulating glucose handling in muscle cells.