Abstract: 140P

LncRNA *Tug1* regulates expression of the mitochondrial calcium uniporter complex in myotubes and cardiomyocytes.

A.J. Trewin, S. Lamon and G.D. Wadley.

Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Burwood, VIC 3125, Australia.

Mitochondria play important roles in multiple biological processes, including the maintenance of cellular Ca^{2+} homeostasis. Elevated cytosolic Ca^{2+} levels are buffered by mitochondria via the mitochondrial calcium uniporter complex (mtCU). The mtCU consists of pore-forming proteins including the mitochondrial calcium uniporter (MCU), and regulatory proteins such as mitochondrial calcium uptake proteins 1 and 2 (MICU1/2). The stoichiometry of these proteins influences the sensitivity to Ca^{2+} and activity of the complex. However, the factors that regulate their gene expression remain incompletely understood. Long non-coding RNAs (lncRNAs) regulate gene expression through various mechanisms, and we recently identified the lncRNA *Tug1* as a modulator of mitochondrial and myogenic transcriptional pathways in skeletal muscle (*1*). In particular, we found that *Tug1* affected the expression of genes that encode mtCU proteins. To further explore this, we knocked down *Tug1* (*Tug1* KD) in C2C12 mouse and L6 rat myotubes as well as H9c2 rat cardiomyocytes using antisense LNA oligos. In all cell lines, *Tug1* KD increased *Mcu* and *Micu1/2* gene expression and increased MCU and MICU2 protein expression. To understand the underlying factors responsible for this effect, we measured phosphorylation of Ca2+/calmodulin-dependent protein kinase II (CaMKII) and its downstream target cAMP Response Element-Binding protein (CREB), a transcription factor known to promote *Mcu* gene expression (*2*). In H9c2 cardiomyocytes, *Tug1* KD attenuated the increase in CAMKII and CREB phosphorylation in response to ionomycin, a Ca^{2+} ionophore. In C2C12 myotubes, *Tug1* KD led to increased pCREB under basal conditions, consistent with the increased mtCU gene and protein abundance. Together, these preliminary data suggest that *Tug1* modulates mtCU expression via a yet to be identified mechanism that may involve CAMKII and CREB. Further studies will also investigate the functional consequences of *Tug1* mediated regulation of MCU on mitochondrial Ca^{2+} uptake, cellular Ca^{2+} handling and the implications for skeletal and cardiac muscle function.

1. A. J. Trewin *et al.*, Long non-coding RNA Tug1 modulates mitochondrial and myogenic responses to exercise in skeletal muscle. *BMC Biol* **20**, 164 (2022).
2. S. Shanmughapriya *et al.*, Ca2+ signals regulate mitochondrial metabolism by stimulating CREB-mediated expression of the mitochondrial Ca2+ uniporter gene MCU. *Science signaling* **8**, ra23 (2015).