Metabolic reprogramming in skeletal muscle stem cells

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While for years considered an innocent bystander in stem cell biology, metabolism has recently taken centre stage, where it has been found to be intricately linked to the generation of new biomass to support high rates of proliferation, and epigenetic changes associated with the processes of lineage commitment, specification and self-renewal (Ryall, 2013). Of particular importance to these processes is the glycolytic pathway. The breakdown of glucose provides both cellular energy in the form of ATP and intermediate metabolites essential for the generation of new nucleotides (via the pentose cycle), phospholipids and amino acids during proliferation (Koopman, Ly & Ryall, 2014; Lunt & Vander Heiden, 2011).

Differential splicing of the muscle isoform of the enzyme pyruvate kinase (*Pkm*) at exons 9 and 10 has been found to be an important regulator of the decision to shunt glycolytic intermediates for breakdown to acetyl-CoA (which will enter the mitochondria and the TCA cycle), or to instead enter the pentose cycle to produce new biomass for cell growth (Ryall, 2013). Inclusion of exon 9 produces PKM1, which catalyzes the dephosphorylation of phosphoenolpyruvate (PEP), and promotes the entry of pyruvate into the mitochondria for conversion to acetyl-CoA. In contrast, exon 10 inclusion produces the PKM2 splice isoform which has a reduced affinity for PEP, and leads to the build-up of glycolytic intermediates available for entry into the PPP. Using both genetic and pharmacologic approaches we have investigated the role of PKM2 in the processes of proliferation and differentiation of C2C12 myogenic cells.

Using plasmid DNA, overexpression of *Pkm2* in proliferating C2C12 cells was found to decrease the extracelluar acidification rate (ECAR, a measure of cellular glycolysis) without altering the rate of oxygen consumption (OCR, a measure of mitochondrial oxidative activity). The decrease in ECAR observed following *Pkm2* overexpression was associated with a significant increase in the rate of proliferation, and delayed differentiation (as evidenced by reduced expression of the differentiation marker myogenin). In contrast, specific inhibition of PKM2 activity with the pharmacological agent shikonin led to a decrease in the rate of proliferation and an increase in the rate of differentiation.

These results place *Pkm* splicing and metabolism squarely at the intersection between proliferation and differentiation in myogenic cells. Future studies will be focussed on manipulating *Pkm* splicing and PKM2 activity *in vivo* to improve skeletal muscle regeneration following injury.

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Supported by the Australian Research Council