Muscle stem cell self-renewal is regulated *via* innate cell metabolism and the extracellular environment

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Skeletal muscle has a remarkable capacity for regeneration; a property conferred by a resident population of skeletal muscle stem cells (MuSCs). In healthy, adult skeletal muscle MuScs exist in a quiescent state outside of the cell cycle. In response to injury these cells rapidly undergo a process of activation, proliferation and differentiation to repair the damaged muscle. Importantly, a small population of these cells return to quiescence to repopulate the local MuSC pool (Dumont *et al.*, 2015). Recent studies in cancer, developmental and stem cell biology have identified the extracellular metabolic environment and local carbohydrate availability as playing a key role in directing changes associated with stem cell self-renewal, lineage specification and the processes of proliferation and differentiation (Ryall *et al.*, 2015). The aim of this study was to investigate the link between the extracellular environment and the processes of self-renewal and myogenic commitment in MuSCs.

Initially, we performed single cell whole transcriptome sequencing (scRNAseq) on freshly isolated MuSCs (quiescent) and MuSCs that had been cultured for 96hrs in growth media (active). Using a t-Distributed Stochastic Neighbor Embedding (tSNE) approach we identified a single clear cluster of quiescent MuSCs. In contrast, active MuSCs were found to localize to two clusters, one overlapping with the quiescent MuSC cluster (self-renewal), and one distinct cluster (committed myogenic progenitors). By analyzing the gene-ontology of these clusters we identified differences in the innate metabolic gene signature of quiescent and self-renewed MuSCs, and myogenic progenitor cells, with the latter demonstrating an enrichment of genes associated with carbohydrate metabolism and the biosynthesis of acetyl-coA.

To better understand the role of metabolism on the process of MuSC self-renewal *versus* myogenic commitment we cultured freshly isolated MuSCs in growth media containing 25 mM glucose or 10 mM galactose. Using a Seahorse XF Bioanalyzer we found that MuSCs cultured in galactose had a reduced metabolic capacity when compared to cells cultured in glucose. These results demonstrate the importance of the extracellular environment in the regulation of innate cell metabolism. To determine the effect of altered innate metabolism on MuSC lineage progression we cultured single fibres from mouse EDL muscles in either glucose or galactose based growth media for 0-96 h. Single fibres cultured in galactose maintained a higher proportion of Pax7+ MuSCs throughout the 96 h, compared to single fibres cultured in glucose ($P<0.05$). A concomitant decrease in the proportion of committed progenitors (MyoD+ cells) was observed at all time points analysed.

These results provide the first clear evidence that the extracellular metabolic environment plays an essential role in regulating MuSC lineage commitment and self-renewal. These results may provide a novel strategy for the *ex vivo* expansion of MuSCs for use in stem cell therapies and have important implications for skeletal muscle regeneration in the context of metabolic diseases.

Dumont NA, Wang YX & Rudnicki MA. (2015) Intrinsic and extrinsic mechanisms regulating satellite cell function. *Development* **142**: 1572-1581.

Ryall JG, Cliff T, Dalston S & Sartorelli V. (2015) Metabolic reprogramming of stem cell epigenetics. *Cell Stem Cell* **17**: 651-662.