Despecification of myogenic C2C12 cells *via* metabolic reprogramming

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Skeletal muscle contains a resident population of muscle stem cells (MuSCs) which are responsible for the high regenerative potential of this tissue. In response to trauma or damaging events, MuSCs, which normally exist in a quiescent state, enter the cell cycle and undergo specification to the myogenic lineage. This is followed by several rounds of proliferation and differentiation; processes that are carefully regulated through the actions of the transcription factors MyoD, Myf5, MyoG and MRF4 (Yin *et al.*, 2013).

Metabolism has recently been proposed to regulate the identity of stem cells and several transcriptomic studies of MuSCs have suggested a metabolic shift towards glycolysis in proliferating compared with quiescent MuSCs (Fukada *et al.*, 2007, Liu *et al.*, 2013). Therefore, we hypothesized that by metabolically reprogramming previously committed myogenic cells back to an oxidative metabolism we could reverse the process of myogenic specification. To this end, C2C12 myogenic cells were cultured in either standard growth medium containing 25 mM glucose (CON) or one of two reprogramming media; 5 mM glucose (LG), or 10 mM galactose (GAL). Following 24 hours culture, analyses of cellular bioenergetics revealed that both LG and GAL groups had decreased basal levels of glycolysis and elevated levels of oxidative phosphorylation (OXPHOS) compared to CON.

Culturing cells in LG or GAL was associated with elevated rates of proliferation, as demonstrated by a significant decrease in the mean doubling time compared to CON (CON: 13.8±0.55 h; LG: 12.7±0.50 h; GAL: 12.8±0.38 h, $P<0.05$). In addition, LG and GAL groups demonstrated elevated levels of bromodeoxyuridine incorporation, indicating an increased proportion of cells in the S-phase of the cell cycle. These results were supported by flow cytometry data for propidium iodide staining demonstrating an elevated proportion of GAL cultured cells in the G2/M phase of the cell cycle and the S phase of the cell cycle (10% and 5% respectively). Importantly whole transcriptome sequencing followed by downstream analyses of gene ontology revealed a decrease in the specification of C2C12 cells to the myogenic lineage, including significant downregulation of *myod1* and *myf5* following 24 hours culture in GAL and to a lesser extent in LG, compared to CON ($P<0.05$). Furthermore we observed an increase in expression of genes associated with the positive regulation of cell cycling and macromolecule biosynthesis in C2C12 cells cultured in GAL or LG compared with CON ($P<0.05$). Western immunoblotting confirmed a decrease in the protein levels of the master myogenic regulator MyoD in C2C12 cells cultured in GAL ($P<0.05$).

These data provide strong support for a link between metabolism and lineage specification and commitment. In the context of MuSCs, culturing cells in a reprogramming medium that causes preferential utilisation of OXPHOS was accompanied by despecification and an increased rate of proliferation. These exciting results provide important insight into the role of metabolism in the regulation of cell identity.

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