Despecification of myogenic C2C12 cells via metabolic reprogramming

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Skeletal muscle plays an essential role in movement, insulating and protecting the internal organs, thermogenesis, as well as acting as a major storage site for metabolites such as glucose. As such any impairment to the structure or function of skeletal muscle (as with the muscular dystrophies, cancer cachexia or age-related muscle wasting) can lead to increased morbidity and mortality (Schertzer *et al.*, 2007). Fortunately skeletal muscle has a large capacity for repair and regeneration due to the presence of a resident population of stem cells; the satellite cell (SC). Given that SCs play an integral role in skeletal muscle regeneration they have become an attractive therapeutic target for autologous transplantation, but to date, this has proven problematic, with 95-99% of transplanted cells dying within days of treatment (Beauchamp *et al.*, 1999). Interestingly a number of studies have shown that freshly isolated SCs exhibit greatly enhanced survival following transplantation; attributed to the reduced expression of the myogenic specification and differentiation factor MyoD1, and elevated expression of the ubiquitous SC transcription factor Pax7 (Smythe & Grounds, 2001).

In the present study we utilized a novel process of 'metabolic reprogramming' to reverse C2C12 myogenic specification. Specifically, we hypothesised that selective *ex vivo* metabolic reprogramming of C2C12 cells to an oxidative phenotype, would lead to reduced expression of MyoD1 and increased markers of 'stemness' such as Pax7. Metabolic reprogramming was induced by culturing proliferating C2C12 cells in growth media containing either high glucose (25 mM, HG), low glucose (5 mM, LG) or galactose (10 mM, Gal). We used a Seahorse bioanalyzer to analyze metabolic reprogramming, and observed that HG cells predominantly generated ATP *via* glycolysis; Gal cells mainly utilized oxidative phosphorylation; and cells cultured in LG used both processes at an intermediate level. Interestingly a comparison of growth rates between the three groups showed that cells cultured in either LG or Gal exhibited a significant decrease in mean doubling time compared to HG cells (LG: 12.7±0.50 h, Gal: 12.8±0.38 h, HG: 13.8±0.55 h, $P<0.05$). Importantly, incubation in Gal was associated with a 15-fold increase in Pax7 expression ($P < 0.05$). In contrast, C2C12 cells cultured in LG exhibited a significant decrease in Pax7 expression. Pax7 immunofluorescent (IF) staining of C2C12 cells identified three populations of cells; Pax7hi, Pax7lo and Pax7-. Similar to that observed with Pax7 gene expression, we observed a two-fold increase in the number of Pax7hi cells when cultured in Gal compared to HG. Associated with the observed increase in Pax7 expression was a decrease in the protein expression of the master myogenic regulator MyoD1 ($P < 0.05$).

Taken together these results indicate that culturing cells in Gal based growth media was sufficient to induce metabolic reprogramming to an oxidative phenotype. Furthermore, this process was accompanied by de-specification and increased proliferative capacity of these cells. These exciting results may have important implications for autologous transplantation therapies, and demonstrate a role for metabolism in the regulation of cell identity.

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