Thioredoxin-interacting protein inhibits glycolysis and proliferation of myogenic cells

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Adult skeletal muscle stem cells (MuSCs) are derived from the mesoderm during early development and are responsible for the high regenerative capacity of skeletal muscle. Adult MuSCs primarily exist in a quiescent state and express the paired homeobox protein Pax7. In response to injury, MuSCs leave the quiescent state and undergo activation and myogenic specification (determined *via* the expression of MyoD), proliferation and eventually differentiation, fusion and maturation (Brack & Rando, 2012). The proximity of MuSCs to blood vessels and the energetic demands imposed during the activation and proliferation of MuSCs suggest a potential regulatory role of metabolism in MuSC biology during these processes. Whole transcriptome analyses of quiescent and proliferating MuSCs have previously identified an enrichment of genes encoding for glycolytic enzymes, and a decrease in the expression of those known to inhibit glycolysis. One such protein, thioredoxin-interacting protein (TXNIP), is one of the most significantly downregulated genes in proliferating MuSCs, suggesting that it may play a role in the regulation of proliferation in MuSCs.

We first characterized the expression of *Txnip* in proliferating and differentiating C2C12 myogenic cells, where we observed an increase in both the gene and protein expression as C2C12 cells switched from proliferation to differentiation. To better understand the role of TXNIP in myogenesis we used transient transfection to overexpress a TXNIP-eGFP fusion protein in proliferating C2C12 cells. Transient transfection (0.1-5µg DNA per 1×10^5 cells) led to a dose-dependent increase in the protein expression of TXNIP in proliferating C2C12 cells.

Overexpression of TXNIP in proliferating C2C12 cells led to a 22 and 26% decrease in basal and maximal glycolysis ($P<0.05$) in the absence of any detectable change in oxygen consumption. This TXNIP mediated decrease in glycolysis was associated with an inhibition of cell proliferation, with the mean doubling time (T_d) increasing from T_d=12.5h in control cells to $T_d=\infty$. The decrease in proliferation was confirmed *via* a 66% decrease in BrdU staining (an indicator of the proportion of cells in S-phase) in TXNIP-eGFP cells.

As metabolism has previously been proposed to regulate the identity of stem cell populations (including MuSCs, Ryall, 2013) we investigated the relative levels of myogenic commitment in TXNIP-eGFP cells. Overexpression of TXNIP was found to lead to a decrease in expression of both the master myogenic regulator MyoD (37%, $P<0.05$) and the upstream transcription factor paired homeobox 7 (Pax7, 56%, $P<0.05$), suggesting that increasing levels of TXNIP may lead to either decreased myogenic commitment or precocious differentiation.

These exciting results demonstrate that TXNIP inhibits both proliferation and myogenic commitment in C2C12 cells. Furthermore, these results support previous findings identifying TXNIP as a negative regulator of glycolysis (Stoltzman *et al.*, 2008). We believe that a better understanding of the link between metabolism and MuSC biology may lead to the identification of novel therapeutic targets for muscle injuries and numerous muscle wasting disorders.

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