Myc Regulates a Transcriptional Program that Stimulates Glutaminolysis

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Introduction: High levels of both glucose and glutamine consumption are required for rapid proliferation of many cell types including stimulated lymphocytes and most cancers. Recent studies have demonstrated that excessive glucose consumption and lactate formation occur in transformed cells with mutations in phosphoinositol 3-kinase (PI3K) and its downstream effector AKT. However, studies in our lab have demonstrated that this signaling pathway does not regulate glutaminolysis (1). The oncogenes directly involved in the control of glutamine metabolism are currently under investigation. In this work, the role of myc in regulating the transcriptional control of glutaminolysis was examined. Two different models were used. In the first, immortalized mouse embryo fibroblasts (MEF) with inducible myc activity were compared un-induced controls. In the second, human glioma cells (SF188) with naturally high myc levels were transfected with short-hairpin RNA (shRNA) against myc. ^{13}C and ^{14}C glutamine were used to determine the impact of myc in regulating pathways that consume glutamine.

Methods: SV40-immortalized MEF cells stably transfected with MycER (a gift from Drs. AT Tikhonenko and R.A. Amaravadi of U. of Pennsylvania) were cultured in DMEM, 10% fetal bovine serum (FBS), and Pen/Strep, 25 mM glucose, and 6 mM L-glutamine. To activate MycER protein (a construct of Myc and the estrogen receptor), cells were incubated with 200 nM 4-hydroxytamoxifen (4-HT) for 24 h. SF188 (human glioma, grade 4) cells were cultured as described previously (2). To knock down myc expression in SF188 cells, they were transduced with shRNA by a lentivirus. A control culture was transduced with a non-functional shRNA.

Metabolic tracing experiments were conducted in DMEM without glutamine and with 10% dialyzed FBS, that was supplemented with either un-enriched glutamine or with [U-$^{13}C_5$]glutamine (Isotec, St. Louis, MO) at 3 mM. Cells were grown to ~70% confluency in 225 cm^2 flasks and cultured with the ^{13}C enriched medium for 6 h. The extracellular medium was analyzed directly without concentration in an 89-mm bore, 400 MHz spectrometer with a 20-mm broad band probe (Varian Inc, Palo Alto, CA). Spectral parameters were: PA = 60º, TR = 3s, 16384 points, 25000Hz SW, bilevel ^1H WALTZ16 decoupling, and 1 Hz exponential line broadening. Carbon-3 of lactate derived from glutamine was a doublet (21.5 and 20.3 ppm) due to ^{13}C coupling with carbon-2, while lactate derived from natural abundance ^{13}C of glucose, produced a singlet at 20.9 ppm. Gas Chromatography–Mass Spectrometry (GC/MS) was conducted as described previously (4) to determine glutaminase activity with [γ-^{15}N]glutamine (Cambridge, Andover, MA). To determine incorporation of glutamine into protein, SF188 cells were cultured in medium supplemented with 0.01% [U-$^{14}C_5$]glutamine for 4 hours; a detergent cell extract was analyzed with a scintillation counter (PerkinElmer, Waltham, MA). Expression of Myc-targets was determined with real-time PCR (Applied Biosystems, Foster City, CA).

Results and Discussion: Immortalized MycER MEF cells treated with 4-HT for 24 hours had increased levels of the transcripts for the glutamine transporter ASCT2, glutaminase ($P < .005$) (which glutamine to glutamate), and lactate dehydrogenase-A (LDH-A) ($P < .005$). These increases in mRNA levels correlated with enhanced functional activity as evidenced by increases in glutamine uptake ($P < .05$) and glutaminase flux ($P < .005$) (by ^{15}N GC/MS). ^{13}C NMR spectroscopy indicated that the fraction of lactate derived from glutamine increased by 96% ($P < .05$). This was evident as an increase in the satellites on the lactate-3 resonance (Figure 2). Despite increasing glutamine consumption, Myc induction did not increase the rate of proliferation of MEF cells.

The opposite experiment was conducted in SF188 cells, in which normally high myc levels were knocked down with shRNA. shMYC cells had an approximately 80% reduction in Myc protein level. This led to a statistically significant reduction in glutamine consumption ($P < .01$) and ammonia production ($P < .05$). With quantitative RT-PCR, shMYC cells were found to express significantly lower levels of the high affinity glutamine transport proteins ASCT2 and SN2 ($P < .01$) without significant changes in the level of expression of a control transcript EIF1A. Furthermore, when Myc antibodies were used to perform chromatin immuno-precipitation (ChIP), Myc was found to selectively bind to the promoter regions of both ASCT2 and SN2. Consistent with these findings, ^{13}C NMR detected a 42% reduction in the amount of lactate derived from [U-$^{13}C_5$]glutamine, relative to that derived from glucose. (Note that protein synthesis, detected with ^{14}C, was a relatively minor fate of glutamine in SF188 as shown in Figure 1.)

Conclusions: The results support the hypothesis that myc specifically upregulates the glutaminolytic pathway, which is essential for rapid cell proliferation. Myc targets include glutamine transporters, glutaminase, and lactate dehydrogenase, all of which are essential for rapid glutamine catabolism.

References:

(1) Wise et al. PNAS 105:18782-18787 (2008).
(2) Mancuso et al. MRM 54:67-78 (2005).
(3) Thomas-Tikhonenko A, et al. Cancer Res 64:3126 (2004).
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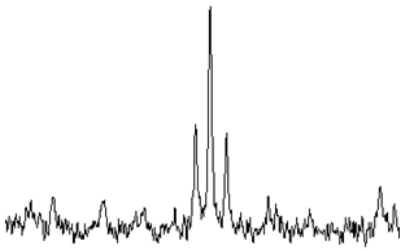

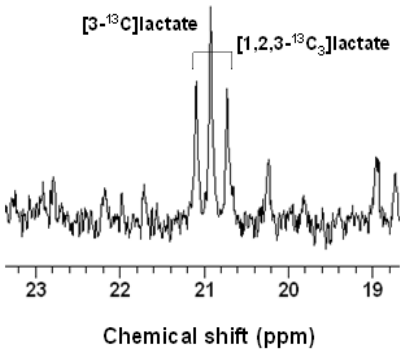

Figure 1. Top – MycER MEF cells without activation, Bottom – MycER MEF cells with 4-HT activation of myc

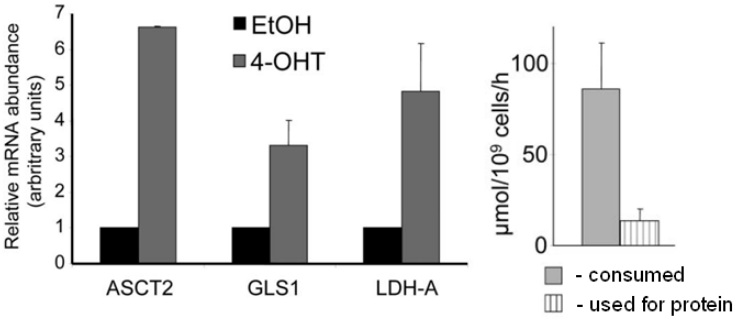

Figure 2. Left – Myc activation in MycER MEF's increases glutamine transport and LDH
Right – ^{14}C data showing glutamine consumed is not used for protein synthesis