Hypoxia Enhances De Novo Fatty Acid Synthesis from Glutamine and Reduces De Novo Fatty Acid Synthesis from Glucose in TSC2-/- Mouse Fibroblasts

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Target Audience: This work should be of interest to basic scientists who wish to understand the impact of hypoxia on *de novo* lipogenesis in cultured cancer cells, a pathway that is currently being examined as a potential therapeutic target.

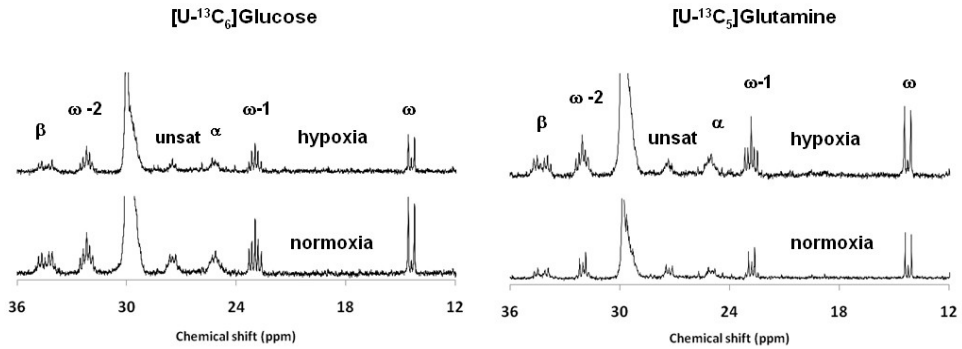
Purpose: The goal of this work was to examine the role of hypoxia on *de novo* lipogenesis in mouse fibroblasts with a homozygous TSC2 (tuberous sclerosis protein 2) deletion. TSC2 is an important component of the akt/m-tor signaling cascade, a cascade that is frequently overactive in many cancers[1]. Normally, Akt phosphorylates TSC2, which suppresses mTOR activity, an important nutrient sensing protein involved in cell growth. TSC2-/- mouse fibroblasts thus serve as a general model for cancers with elevated Akt/mTOR activity.

Methods: TSC2-/- fibroblasts were grown in DMEM supplemented with 10% dialyzed fetal bovine serum. The glucose and glutamine levels were 12 and 3 mM, respectively. For labeling studies, un-enriched glucose or glutamine were completely replaced with [U-$^{13}C_6$]glucose (Cambridge Isotopes, Andover, MA, USA) or [U-$^{13}C_5$]glutamine (Isotec, St. Louis, MO, USA). Labeling studies were conducted with cultures grown to 5 x 10^6 in 225 cm^2 T-flasks. The flasks were incubated under either normoxic (21% oxygen) or hypoxic (1% oxygen) conditions. The duration of labeling was ~16 hours, during which cellular proliferation continued. At the end of the experiment, cultures were washed with PBS, trypsinized, and re-washed with PBS containing 1% fatty-acid-free bovine serum albumin to remove traces of extra cellular lipids. Total cellular lipids were extracted with a standard chloroform/methanol procedure[2] and re-disolved in 0.35 ml of $CDCl_3$. The cellular extracts were analyzed in 5-mm Shigemi (Alison Park, PA, USA) tubes with a broad-band 5-mm NMR probe on a 9.4 T Varian spectrometer (Palo Alto, CA, USA). Acquisition parameters were: 60^o pulse width, 3 s relaxation delay, 25000 Hz SW, bi-level WALTZ16 decoupling with continuous proton decoupling. Nuts NMR (Acorn NMR, Livermore, CA, USA) was used for resonance area determination. The labeling experiments were performed in triplicate.

Results: Under normoxia, TSC2-/- cells had a doubling time of ~24 h; under hypoxia, the doubling time was reducing by ~10%. This result is consistent with those observed for other cultured cancer cells in our lab. Typical ^{13}C spectra are show in the figure below. The primary resonances detected were those for fatty acyl carbons[3]. The triplet at 14.5 ppm represents the terminal CH_3 (ω) of all fatty acyl groups in cellular lipids, with the center peak arising from the natural abundance carbons and the two satellites arising from the ^{13}C-^{13}C coupled carbons produced from either uniformly labeled glucose or glutamine. The natural abundance ω resonance was used as an internal standard for the entire spectrum. Other carbons labeled were ω - 1 and ω - 2 (one and two carbons from the methyl terminus), the α and β carbons near the ester carbons, and a large peak at 30 ppm associated with much of the remaining fatty acyl carbons[3]. The carbons at ~28 ppm are associated with those coupled to alkene groups. The alkene groups themselves were detected at ~130 ppm (not shown). Over the course of the 16 h normoxic experiment, [U-$^{13}C_6$]glucose produced an 18 +/- 2% enrichment in the ω carbons. This value was reduced to 7.0 +/- 0.5% by hypoxia. For glutamine, the enrichments were 7.3 +/- 0.4% and 14 +/- 1% for normoxia and hypoxia, respectively. For alkene carbons, the enrichments for normoxia and hypoxia from glucose were 17 +/- 1.5% and 4.6 +/- 0.8% and from glutamine were 7.5 +/- 0.3% and 7.8 +/- 0.2%, respectively

Discussion: The results demonstrate that hypoxia causes a marked reduction of *de novo* synthesis of saturated fat from glucose and a marked increase of *de novo* synthesis of saturated fat from glutamine. Hypoxia also caused a large reduction in synthesis of unsaturated fat from glucose but had no effect on unsaturated fat synthesis from glutamine.

Conclusions: The results suggest that under hypoxia, glutamine is preferentially used for *de novo* fatty acid synthesis. Additional studies in our lab have demonstrated that this occurs by the non-oxidative reductive carboxylation enzyme isocitrate dehydrogenase (data not shown). These results could provide insight into the design of cancer therapeutics that target *de novo* lipogenesis of solid tumors that have hypoxic regions.



References:

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