

## Both the Glutaminolytic and Reverse Isocitrate Dehydrogenase Pathways are Important for De Novo Lipogenesis from Glutamine in Immortalized Hematopoietic Cells

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**Introduction:** We have been studying *de novo* lipogenesis in mammalian cells because of its fundamental importance in growth and proliferation. Glucose is usually the primary precursor for this process, generating acetyl-CoA to supply the fatty acid synthase complex. However, we recently observed that human glioblastoma cells derive a significant amount of their carbon for lipids from glutamine [1]. We have also observed that immortalized hematopoietic cells (IL-3 DKO) can grow without glucose, provided that a key signaling molecule normally derived from glucose, N-acetylglucosamine (GlcNac), is present in the culture medium [2]. In this study, we examined the role of glutamine in providing carbon for *de novo* lipogenesis in immortalized hematopoietic cells. [3-<sup>13</sup>C] and [5-<sup>13</sup>C]glutamine were used to quantify the flux from glutamine to fatty acyl groups by both the glutaminolytic pathway [3] and the reverse isocitrate dehydrogenase (IDH) pathway [4].

**Methods:** An immortalized hematopoietic cell line derived from *bax*<sup>-/-</sup> *bak*<sup>-/-</sup> mice was used (IL-3 DKO). They require interleukin-3 to grow and proliferate and are resistant to apoptosis under nutrient deprivation [4]. The medium was RPMI 1640 with 10% FBS, pen/strep, 10 mM HEPES, 50 μM β-mercapto ethanol and 3 ng/ml IL-3 (BD Biosciences). Prior to labeling experiments, cells were washed and suspended at 3 x 10<sup>5</sup>/ml in medium without glucose but with 3 mM glutamine for 24 h (37°C). Subsequently, either nothing, 15 mM glucose or 15 mM GlcNac was added to the medium. The glutamine was either unenriched, 99% enriched at carbon 3 or 99% enriched at carbon 5 (9 different conditions). All <sup>13</sup>C glutamine was obtained from Isotec (St. Louis, MO). In a separate experiment, cells were cultured with 15 mM N-acetyl-D-[U-<sup>13</sup>C<sub>6</sub>]glucosamine (Omicron Bio, South Bend, IN) to ensure that this compound was not serving as a catabolite. After 24 h in the <sup>13</sup>C labeled medium, cells were recovered by centrifugation and extracted with a chloroform/methanol procedure [5]. Extracts were examined in CDCl<sub>3</sub> with a CH<sub>2</sub>Cl<sub>2</sub> quantitation standard on a 9.4 Tesla NMR system (Varian, Palo Alto, CA). A 5-mm broad band probe was used for <sup>13</sup>C spectroscopy with 60° pulses, bilevel WALTZ16 <sup>1</sup>H decoupling, 3000 ms TR, 32K points, 30000 Hz spectral width, and 10000 transients. <sup>1</sup>H NMR spectroscopy was used to determine total lipid levels. A 90 pulse was used (64K points, 4000 Hz spectral width) with only one transient. After exponential apodization, peak areas were determined with Nuts NMR (AcornNMR, Livermore, CA).

**Results:** Cells cultured with 15 mM GlcNac and no glucose increased in volume by ~20%. A corresponding increase in total protein was observed, indicating that the change was in fact growth and not just cell swelling. In the lipid extract spectra below, the lower-most trace shows the natural abundance <sup>13</sup>C level. The primary resonances of interest are those for the three terminal carbons of all phospholipids and triglycerides (ω, ω-1, and ω-2) and the combined resonances for the carbons distant from any specific region (-CH<sub>2</sub>-) [1]. [5-<sup>13</sup>C]glutamine did not increase any of the resonances significantly. This result indicates that essentially no flux through the reverse IDH pathway contributed to lipogenesis [1]. In contrast, the <sup>13</sup>C level for all resonances increased by 2.1 fold when [3-<sup>13</sup>C]glutamine was used, demonstrating that the glutaminolytic pathway was very important for lipogenesis [3]. For growth with glucose, the cell volume increased by ~30%, and the cell number increased with a doubling time of ~36 hours. The contribution of the two glutamine consuming pathways was approximately equal (see the histogram). For growth without GlcNac or glucose a ~20% reduction in cell volume was observed; [3-<sup>13</sup>C]glutamine contributed to lipogenesis, perhaps to maintain membrane integrity. Examination of the extracellular medium during incubation with 15 mM N-acetyl-D-[U-<sup>13</sup>C<sub>6</sub>]glucosamine indicated that this substrate was not catabolized. This result is consistent with LC/MS findings for this cell line[2].

**Discussion:** The results demonstrate that glutamine is an important substrate for lipogenesis and that in the absence of glucose, the more energy efficient pathway of glutaminolysis is used predominantly. It generates considerable amounts of ATP in the production of pyruvate, which can be further oxidized or used to make acetate. The reverse IDH pathway is inherently inefficient because carboxylation of alpha-ketoglutarate consumes ATP. It is used by SF188 glioblastoma cells [1] perhaps as a rapid source of acetyl-CoA, at the expense of energy efficiency.

**Conclusions:** The results demonstrate that IL-3 DKO cells produce lipid from glutamine and can use the glutaminolytic and reverse IDH pathways for this process. However, in the presence of glucose, the metabolic pattern is significantly different from that observed for myc-driven glioblastoma cells (SF188), perhaps because they grow more slowly and are not as glutamine dependent.

### References:

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