De Novo Lipogenesis from Glutamine in Human Glioma Cells

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Introduction: Rapidly growing cancer cells require high rates of phospholipid biosynthesis for the formation of new membranes. Although the fatty acyl (FA) component of phospholipids can be derived from serum lipids, glucose is used for *de novo* FA synthesis. Acetyl-CoA units are produced in the mitochondrion and are shuttled to the cytoplasm as citrate (carbons 4 and 5) [1]. They are subsequently condensed and reduced (with NADPH) in the fatty acid synthase complex during long chain FA elongation. One hypothesis for why glucose is used for lipogenesis is that the rate of uptake of intact fatty acids from the extracellular environment is not sufficiently rapid to sustain the high rate of growth. This energetically wasteful form of phospholipid biosynthesis is analogous to the wasteful conversion of glucose to lactate, which rapidly produces ATP but at a very low yield. Inhibition of de novo FA synthesis has been proposed as a therapeutic strategy in cancer [2]. An improved understanding of the pathways involved in *de novo* lipogenesis, especially in differences between normal and cancerous cells, could greatly advance the development of new therapeutics. In this work, FA synthesis in cultured human glioma cells was examined with ^{13}C NMR spectroscopy.

Methods: SF188 cells (human glioma, grade 4, UCSF) were cultured in DMEM that contained 25 mM glucose, 4 mM glutamine, 25 mM HEPES and 10% fetal bovine serum. For ^{13}C experiments, cells were grown to ~30% confluency in 225 cm^2 T-flasks and all of the unlabeled medium was removed and replaced with medium containing enriched substrates (Isotec, St. Louis, MO) as detailed below. The glucose and glutamine concentrations were 15 mM and 3 mM, respectively. The duration of cell labeling was 24 hours or 0.89 cell doublings. Half of the medium in the flask was replaced after 16 hours (with the same ^{13}C labeled medium) to prevent nutrient levels from dropping below the physiologic range. At the end of the experiment, cells were washed with PBS and lipids were extracted with a standard procedure [3].

A 400 MHz spectrometer (Varian Inc. Palo Alto, CA) and a 5-mm broad band probe were used to acquire NOE-enhanced ^{13}C spectra. Acquisition parameters were: PA = 60°, ^1H decoupling = WALTZ16 (bilevel), TR = 3000 ms, 32K points and spectral width = 30000 Hz. Free induction decays were zero filled to 64K points and apodized with exponential multiplication (1.5 Hz). Resonances areas were determined with Nuts NMR (AcornNMR, Livermore, CA).

Results and Discussion: A series of extract spectra acquired for cells grown with either unenriched glutamine, [3-^{13}C]glutamine, [5-^{13}C]glutamine or [U-$^{13}C_5$]glutamine (all with unenriched glucose) are shown in the left figure. Natural abundance resonances (bottom spectrum) were detected for the 3 carbons at the FA methyl terminus (ω: 14.5 ppm, ω-1: 23.0 and ω-2: 32.3 ppm). These resonances represent the sum of carbons for all species of FA including palmitic, stearic, oleic, linoleic, etc [4]. The broad peaks at 30 ppm are the overlapping resonances of many FA carbons distant from unsaturated bonds, esters, etc. In the [3-^{13}C]glutamine spectrum, all of the resonances are enhanced by approximately a factor of 2. This substrate labels both carbons of acetyl-CoA equally through the standard glutaminolytic pathway (glutamate, α-ketoglutarate, succinate, malate, pyruvate) [1]. Resonances for the α (34.0 ppm) and β (25.3) ppm carbons of FA are apparent, as is the resonance for the carbon immediately adjacent to unsaturated bonds at 26.0 ppm. In the [5-^{13}C]glutamine spectrum, the ω-1, β and $-CH_2-$ resonances are strongly enhanced (but not ω or ω-2), indicating that ^{13}C was transferred to only C-1 of acetyl-CoA [1]. This can not occur by the glutaminolytic pathway [1], within which C-5 of glutamine is lost, but is believed to be the result of flux from α-ketoglutarate to citrate via isocitrate [5]. This pathway has been shown to be important for *de novo* lipogenesis in adipocytes [5]. In the [U-$^{13}C_5$]glutamine spectrum, all the FA resonances are enhanced and complex ^{13}C-^{13}C multiplets are present. The coupling constants are ~35 Hz. To quantify the ^{13}C fraction enrichment in $-CH_2-$ and CH_3 FA, both ^{13}C and ^1H (an indicator of total lipid) peak areas were compared to an internal standard peak of unenriched CH_2Cl_2. The fractional enrichments (mol ^{13}C FA/mol total FA) are summarized at the right of each spectrum of the figure on the left. The value for the natural abundance spectrum was slightly high (1.4%) and should have been 1.1%. C-5 of glutamine clearly contributes much more to de novo lipogenesis than does C-3. With label at both C-4 and C-5 of glutamine, the fractional enrichment in FA would probably have been 12% [(7.0-1.1%) x 2]. Given that 0.89 cell doublings occurred, ~27% of the *de novo* carbon was derived from C-4 and C-5 of glutamine and ~3% from C-2 and C-3. One reason the contribution of [3-^{13}C]glutamine to *de novo* FA synthesis is low is on conversation to pyruvate, much ^{13}C is lost as lactate.

To directly compare the rate of *de novo* lipogenesis from glutamine to that from glucose, cells were cultured with medium containing both 15 mM [1-^{13}C]glucose and 3 mM [5-^{13}C]glutamine. [1-^{13}C]glucose labels only C-2 of acetyl-CoA and subsequently the ω and ω-2 FA carbons, while [5-^{13}C]glutamine labels C-1 of acetyl-CoA and the ω-1 FA carbon. The results, shown in the figure on the right indicate that the ω and ω-1 intensities are very similar. Because glucose produces two moles of acetyl-CoA for every mole of glucose consumed, the flux of glucose derived acetyl-CoA (one labeled and one unlabeled) is approximately twice that for glutamine. Also, ~5.8% of the [1-^{13}C]glucose is lost in the pentose phosphate pathway [6]. Taking these factors into account, the flux from C-5 of glutamine to lipid is approximately 57% of the flux from C-1 glucose. Given that glucose contributes approximately 60% to de novo lipogenesis (6), C-4 and C-5 of glutamine contributes ~34% of the total flux to acetyl-CoA. This value is comparable to the value of ~27% estimated in the above paragraph. Some or all of the remaining carbon is derived from C-2 and C-3 of glutamine from the glutaminolytic pathway.

Conclusions: The results demonstrate that glutamine is a major source of carbon for *de novo* lipogenesis. Some flux to acetyl-CoA occurs through the standard glutaminolytic pathway, but most is through the direct conversion of alpha-ketoglutarate to citrate via isocitrate.

References:

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