Determination of ^{13}C Labeling Rates in Fatty Acyl Groups from [1,6-$^{13}C_2$]Glucose in Human Glioma Cells

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Introduction: Tumor cells often have elevated levels of enzymes involved in *de novo* fatty acid synthesis to match their high growth rates (1-3). These fatty acids are used to synthesize phospholipids that are essential for the assembly of daughter cell membranes. Inhibition of lipogenic enzymes has been proposed as a therapeutic target and pilot studies in model systems have yielded optimistic results (1,2,4). However, many mechanistic details of tumor lipogenesis are not well understood and elucidation of these details may facilitate the development and use of lipid synthesis inhibitors in cancer therapy. Our initial goal is to delineate the primary metabolic pathways that are used for lipid synthesis in a model human glioma cell line.

Natural-abundance ^{13}C resonances of lipids in tumors can be very broad due to chemical shift anisotropy and possibly long rotational-correlation times (5). However, studies in cultured cells have demonstrated that during labeling with lipogenic substrates, some fatty acyl groups produce narrow resonances that can be readily quantified (6). The rates of change of these resonances may be directly proportional to the total flux of carbon into specific fatty acyl groups. In this study, we quantified the rate of ^{13}C labeling into specific fatty acyl groups from [1,6-$^{13}C_2$]glucose.

Materials and Methods: SF188 cells (human glioma grade 4, Brain Tumor Research Center, UCSF) were grown with DMEM medium (supplemented with 10% serum and 50 μg/ml gentamicin) in porous collagen microcarriers (Hyclone, Logan, UT). These microcarriers had a mean diameter of 200 μm when fully hydrated and were used in combination with non-porous polystyrene spheres (1:1 volume ratio) inside a 20-mm NMR tube (7). The cells were sustained at physiologic conditions (37 °C, pH = 7.2, dissolved oxygen = 0.2 mM) inside a 9.4T spectrometer (Varian, Palo Alto, CA) (7). ^{31}P spectral parameters were: 60° pulse width, 1000 ms repetition time, 4096 points, and 15000 Hz spectral width. ^{13}C spectra were acquired with 60° pulses, a repetition time of 1200 ms, 4096 points, 25000 Hz spectral width and ^{1}H bi-level WALTZ-16 decoupling. Cells were initially fed DMEM with 10 mM un-enriched glucose while background spectra were acquired. Subsequently, the un-enriched medium was completely replaced with DMEM containing 10 mM [1,6-$^{13}C_2$] glucose (Cambridge Isotopes, Andover, MA). When the extracellular glucose concentration dropped to 4 mM, a steady feed of DMEM with 10 mM [1,6-$^{13}C_2$] was used to maintain the glucose level at 4 mM. For metabolite quantitation, resonance areas were determined with Nuts (Acorn NMR, Livermore, CA, USA). Absolute intracellular concentrations were calculated as described previously (7). Labeling rates were determined with a standard least-squares linear regression and are reported as mean ± standard error.

Results: ^{13}C results for an actively replicating culture (7 x 10^8 cells, as estimated from ^{31}P NMR-detected NTP levels (8)) are shown in Figures 1 and 2. The spectrum in Figure 1 was acquired 7 hours after the start of ^{13}C labeling. A substantial amount of label was detected in C-3 of extracellular lactate and alanine. Labeling in C-4 of glutamate, a key reporter molecule for TCA cycle flux, was initially rapid and reached a steady state within approximately 4 hours. Label was also detected in C-3 glutamate and the resonance was a triplet due to ^{13}C-^{13}C coupling with C-4 glutamate. Four resonances associated with fatty acyl groups were detected, including two (30.4 and 29.8 ppm) for -$(CH_2)_n$- distant from non-alkyl groups, one for –CH_2- groups that were two carbons away from the methyl terminus (32.6 ppm) and one for the CH_3- methyl terminus (14.9 ppm). Of these, the first 2 could be readily quantified with 15-min temporal resolution and the third could be quantified with 30-min resolution. The 14.9 ppm was difficult to curve fit and integrate due to its low signal to noise ratio and asymmetry. The rates of accumulation of ^{13}C label into the 30.3, 29.9 and 32.6 ppm resonances were 0.62 ±0.02, 0.20 ±0.01, 0.24 ±0.01 mmol/L-cell/h, respectively. These rates were constant throughout the experiment (Figure 2). The levels of label in fatty acyl groups did not decline after the ^{13}C glucose was removed from the culture medium (data not shown). In contrast, a decline in labeling levels was observed for small metabolites, such as glutamate.

Discussion: The data demonstrate that in cultured human glioma cells, glucose is a significant carbon source for fatty acyl groups of lipids. Labeling was observed within 45 min of the addition of the ^{13}C glucose and continued in a linear manner throughout the time that ^{13}C glucose was present in the medium. The observation that the fatty acyl ^{13}C resonances persisted after the labeled glucose was removed from the culture medium suggests that these groups were permanently incorporated into membrane lipids.

Conclusions: Glucose is used as a lipogenic precursor during cellular proliferation of cultured glioma cells *in vitro*. Future experiments will examine the relative contribution of other nutrients such as glutamine to fatty acyl biosynthesis and will further delineate the metabolic pathways tumor cells use to produce lipids.

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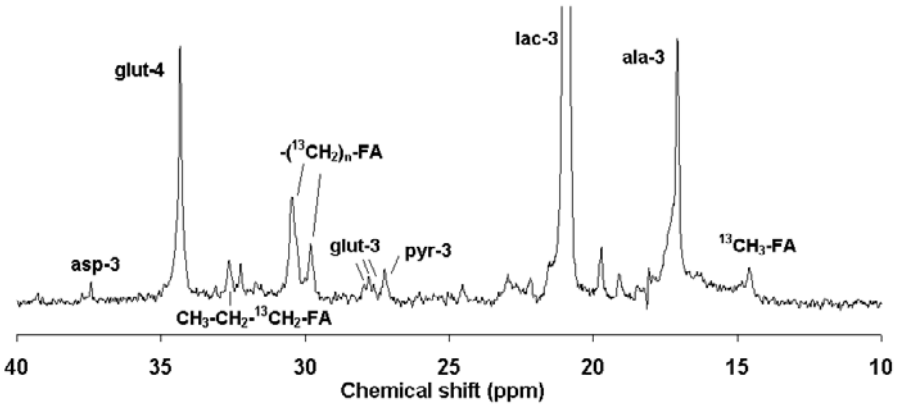

Figure 1. ^{13}C spectrum of proliferating SF188 cells during infusion of [1,6-$^{13}C_2$]glucose. This spectrum was obtained in 30 min. The background was removed by subtraction with a 30-min baseline spectrum. Four resonances were observed for ^{13}C labeled fatty acyl groups: 14.9, 29.9, 30.3 and 32.6 ppm.

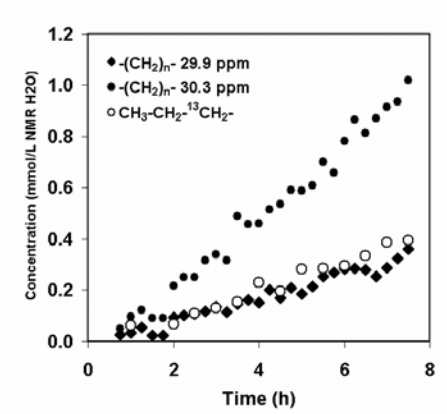

Figure 2. Labeling profiles for ^{13}C labeling in fatty acyl groups from [1,6-$^{13}C_2$]glucose. The addition of labeled glucose began at time zero.