13C NMR studies of lymphoma and melanoma cells in the perfusion bioreactor and in vivo xenografts for flux calculation

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Target audience: Cancer metabolism researchers.

Purpose: To elucidate the detailed mechanism of tumor intermediary metabolism and calculate various fluxes through metabolic pathways in lymphoma and melanoma cells as well as in tumors *in vivo*, and to identify flux changes following various interventions.

Methods: A versatile cell perfusion bioreactor system was constructed that is applicable to both anchorage-dependent and independent cells. A 10 mm NMR tube was employed to prepare the perfusion chamber. For lymphoma cells (anchorage-independent), the WSU-DLCL2 cell line (diffuse large B-cell) was used with an agarose bead encapsulation method.[1] Anchorage-dependent DB1 melanoma cells were.grown on the surface of solid microcarrier beads (Solohill). The culture medium containing 1,6-^{13}C glucose was mixed with 20% O_2, 5% CO_2 and 75% N_2 at 37°C. To monitor and record oxygen consumption rates, fiber optic dissolved oxygen sensors (Ocean Optics) were located before and after the NMR tube along the flow path. Studies were performed on a 9.4 Tesla Varian Inova vertical bore NMR system using a ^{13}C pulse acquire sequence with Waltz ^{1}H decoupling. For in vivo experiments, mice with subcutaneous ~500 mm^3 tumor were studied using a home-built dual tuned ^{1}H/^{13}C loop gap resonator and horizontal bore magnet. 450 mM 1,6-^{13}C glucose in PBS was infused through the tail veins of nude mice (~30 mg) at a variable infusion rate to maintain the blood glucose at a constant level (~18 mM). After ^{1}H MRI scout images, a ^{13}C NMR ISIS voxel was selected followed by a ^{13}C pulse acquire sequence with Waltz ^{1}H decoupling. Animal studies were performed on WSU-DLCL2 lymphoma xenografts and WM983B melanoma xenografts. For treatment of lymphoma cells and xenografts, an mTOR signaling inhibitor rapamycin was administered to cells (200 nM) or animals (10 mg/kg twice a day, four consecutive days) as in our previous ^{1}H MRS study.[2]

Results: Fig 1 shows the time course of ^{13}C NMR spectra of perfused DLCL2 lymphoma cells before and after mTOR inhibitor (rapamycin) treatment. Accumulation of C3-Lac, C3-Ala, C4-Glu and C1-Glycogen with time was clearly visible. All of these metabolites decreased after rapamycin treatment as observed in the bottom figure with the same number of cells (4x10^8). Fig 2 shows the time course of ^{13}C NMR spectra of a DLCL2 lymphoma xenograft (top) and a WM983B melanoma xenograft (bottom). Labeling enrichments of C1-Glc, C6-Glc, C3-Lac, C3-Ala, C4-Glu and C1-Glycogen were observed as in the perfusion experiment. Spectra from the lymphoma xenograft exhibited narrower linewidths than the melanoma xenografts indicating more homogeneity in the lymphoma xenograft. The melanoma xenograft, however, presented higher TCA cycle flux than the lymphoma xenograft as indicated by a bigger net glutamate peaks and smaller lactate production. After treatment with rapamycin, all the lymphoma metabolites except glucose decreased (data not shown here). For the DB1 melanoma cells in the perfusion experiment, serum deprivations, suppressed all the fluxes except glycogen (data not shown here).

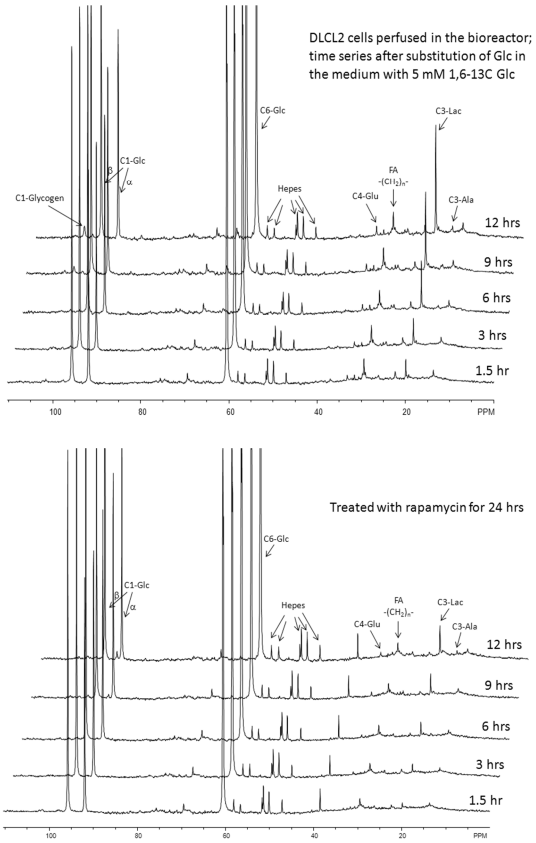

Fig 1. ^{13}C NMR of perfused WSU-DLCL2 cells without (top) and with (bottom) rapamycin treatment.

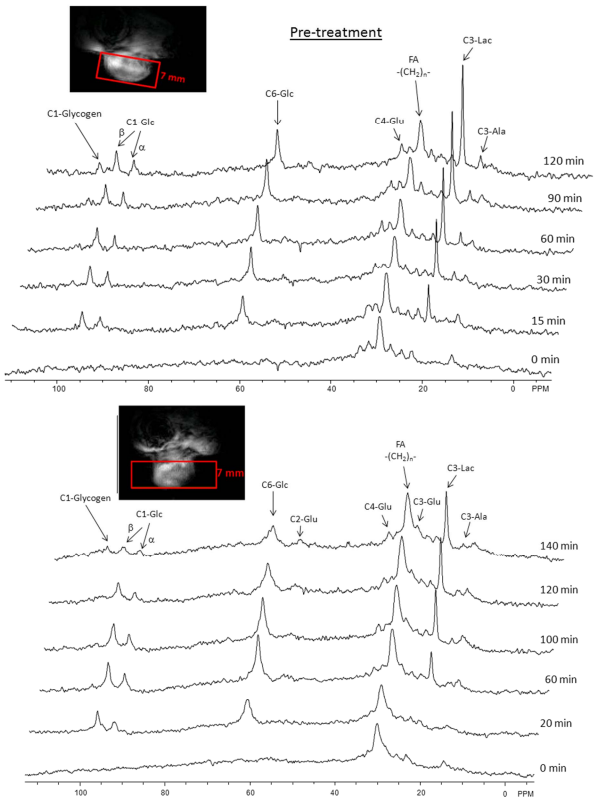

Fig 2. Time course localized ^{13}C NMR of a WSU-DLCL2 lymphoma xenograft (top) and a WM983B melanoma xenograft (bottom).

Discussion: We have shown in our previous study with ^{1}H MRS that lactate is a very early and sensitive marker of mTOR signaling inhibition in various lymphoma cells and xenografts.[2] ^{13}C NMR provides further details of metabolic fluxes through various pathways. We are in the process of calculating various metabolic fluxes (glycolysis, TCA cycle, anaplerosis, glutaminolysis, etc) both in the perfused cells and *in vivo* tumors using the bonded cumomer model.[3] In the tumor, such flux analysis that includes TCA cycle and glycogen synthesis was not performed before nor were well-resolved *in vivo* dynamic ^{13}C spectra previously reported.

Conclusion: We show for the first time that the time course of ^{13}C NMR monitors changes in flux through glycolysis, TCA cycle and glycogen synthesis in lymphoma and melanoma cells both in the perfusion system and in *in vivo* tumor xenografts. Note that we have utilized 1,6-^{13}C glucose without dynamic nuclear polarization. The flux analysis based on these data will provide unprecedented information of tumor metabolic fluxes. These methods can be directly translated into studies of human cancer patients at 3T, 7T or higher fields

References: 1. Bental M and Deutsch C. Magn Reson Med. 1993, 29(3):317-26. 2. Lee SC et al. NMR Biomed. 2013, 26(1):106-14. 3. Shestov AA et al., Adv Exp Med Biol. 2013, 765:265-71.
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