13C MRS/Bioreactor Technique to Study Melanoma: Quantifying Glutaminolysis and de novo Lipogenesis

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Introduction. Bioreactor techniques are critical to the detailed mechanistic study of cancer cell metabolism (1, 2). Modeling of intracellular MRS isotopomer data obtained during perfusion with ^{13}C labeled substrates allows quantitative determination of metabolic fluxes and other metabolic parameters *in living cells* and is a basis for subsequent study of these parameters *in vivo* in rodent xenografts of human cancer and eventually for *in situ* studies in human patients. Dynamic isotopomer modeling takes optimal advantage of biochemical information that can be assessed with NMR spectroscopy by considering all the different isotopomers of metabolites. The goal of the present work was to apply a ^{13}C metabolic "bonded cumomer" (3) modeling approach adapted for fitting Continuous-Stirred Tank (CST) Bioreactor data obtained with ^{13}C labeled substrates, to calculate the extent of the Warburg effect and to quantify *de novo* fatty acid synthesis and glutaminolysis fluxes in DB1 melanoma cells carrying the BRAF V600E mutation.

Methods. The time courses of [3-^{13}C] lactic acid, [4–^{13}C]-, [3–^{13}C]- and [2–^{13}C] glutamate as well as the glutamate-C4d34 doublet, C3d doublet and C2s singlet were monitored. Data were analyzed with a metabolic network model that included glucose and lactate transport, glycolysis, TCA cycle, pentose-phosphate pathway, α-ketoglutarate-glutamate and oxaloacetate-aspartate exchange, pyruvate carboxylase activity, anaplerosis at the succinyl-CoA level, pyruvate recycling through malic enzyme and lactate dehydrogenase activity. Isotope balance equations were derived for every bonded cumomer of order 1,2,or 3 of glutamate, glutamine and aspartate. This resulted in a set of ~150 differential equations that were solved to determine flux through the various metabolic pathways by isotopomer/cumomer analysis (3), Solving a system of differential equations (using the Runge-Kutta algorithm) in terms of bounded cumomers yields time courses for all possible fine structure ^{13}C multiplets of e.g glutamate, glutamine and aspartate. Minimization was performed using the Broyden, Fletcher, Goldfarb and Shanno or Simplex algorithms. Proper mean-square convergence was confirmed by verifying that goodness-of-fit values were close to expected theoretical values. The errors for the parameters derived from data fitting were estimated by Monte Carlo simulations with experimental noise levels. All numerical procedures were carried out in Matlab. Human DB1 melanoma cells were grown in culture on nonporous polystyrene beads and loaded into a bioreactor consisting of an NMR tube together with porous collagen beads (1,2). The cells were perfused with 26 mM [1,6-$^{13}C_2$] glucose at 37^{0}C while the ^{13}C NMR spectrum was recorded with a 9.4 T/89 mm vertical bore Varian Spectrometer.

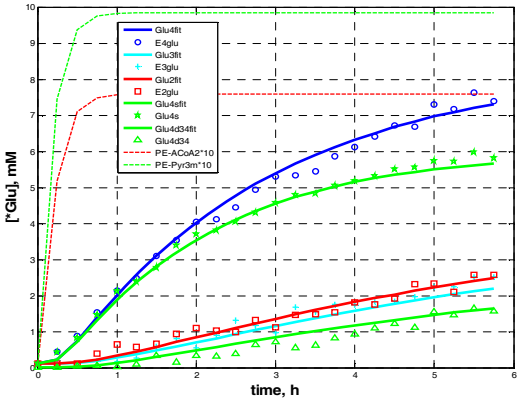

Fig. 1 [1,6-$^{13}C_2$]glc perfusion. Total glutamate C4, C3, C2, doublet C4D34 and C4s singlet labeling time courses. Continuous lines represent the best fits (totally 8 curves, other not shown) to the experimental ^{13}C MRS data. Dashed lines represent predicted Pyr labeling in mitochondrial (red) and cytosolic compartments (green) multiplied by factor 10.

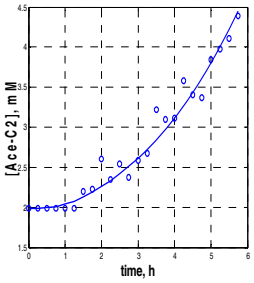

Fig. 1. [1,6-$^{13}C_2$]glc perfusion. Time labeling course of methylene [-$(CH_2)_n$ -] groups of fatty acids. Continuous line represent the best fit.

Results.Figure 1 shows experimental time courses for labeled glutamate obtained during [1,6-$^{13}C_2$] glucose perfusion of melanoma cells cultured in a CST bioreactor. Figure 2 shows data for lipid *de novo* synthesis in proliferating melanoma cells. Metabolic fluxes determined from kinetic curves were (in mmol/Lcell/hr): Ftca=10.5+/-8%, glutaminolysis Fglz=0.0., de novo fatty acid synthesis Ffa=0.85+/-10%. TCA flux and especially anaplerotic exchange flux were high in melanoma cells; fatty acids synthesis was relatively low. The estimated cellular metabolic rate of oxygen consumption ($CMRO_2$) from two data sets was 34+/-2 mmol/Lcell/h compared to the experimental rate 33+/-4 mmol/Lcell/h.

Discussion and Conclusions. The model is validated by excellent agreement between model predicted and experimentally measured values of $CMRO_2$ and mitochondrial+cytosolic glutamate pool size. This ^{13}C metabolic model takes into account the positional labeling as well as the additional information available from ^{13}C the multiplets in ^{13}C spectra. This leads to significantly increased precision in calculated metabolic fluxes and allows estimatation of contributions of different pathways to particular glutamate labeling isotopomers. Also the new cumomer approach significantly reduces the number of kinetic isotopomer equations required to generate a full kinetic model in terms of participating fine structure ^{13}C multiplets and provides a more effective method for investigation of detailed mechanisms of cancer metabolism. Dynamic high-resolution MR spectra are very sensitive to changing/adding biochemical pathways and flux values, and cumomer modeling permits precise checking of the feasibility of assumed general bionetworks and particular metabolic pathways.

References. 1. DeBerardinis RJ, Mancuso A. *et al., PNAS* 2007, 104, 19345 **2.** Shestov AA, Mancuso, A, et al., Oxygen Transport to Tissue 2013, 34, 265, **3**. Shestov AA, Valette J. et al, *Neurochem Res 2012,37, 2388*. **Acknowledgements:** This work was supported by NIH grants 5R01CA129544-02, R01-CA172820