C6 glioma cells depict an active pyruvate recycling system that is redox sensitive

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Introduction. Pyruvate recycling is a well known pathway in the liver, that returns to the pyruvate dehydrogenase and citrate synthase steps, pyruvate or acetyl-CoA skeletons, derived from tricarboxylic acid cycle intermediates. Its physiological relevance appears to be related to its capacity to produce NADPH reducing equivalents, protecting against situations of redox and free-radical stress. Despite its importance, its cellular localization, modulation and physiological significance in the brain remain unclear (1,2). Moreover, even though it has been previously suggested that the activity of this pathway could reach significant values in tumoral cells, no previous studies have addressed this topic in detail (3). On this basis, we report here the presence of pyruvate recycling in cultures of C6 glioma cells and evaluate its dependence on the intracellular redox state.

Methods. C6 glioma cells were grown to confluence in DMEM and incubated (3-48 h, 37 ºC) in Krebs-Henseleit-Buffer (KHB) with 10 mM (U-$^{13}C_3$) lactate or 10 mM (2-^{13}C) pyruvate. Aliquots from the medium (1 mL) were collected during the incubation sequentially and analyzed by high resolution ^{1}H NMR (500.13 MHz, 25 ºC, pH 7.2) and gated broad-band proton-decoupling ^{13}C NMR (125.130 MHz, 25 ºC, pH 7.2) using an AVANCE 500WB NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). ^{1}H or ^{13}C chemical shifts were referred to that of 2,2´-3,3´-tetradeutero-tetrametylsylil propionate (TSP) at 0 ppm or dioxane at 67.4 ppm, respectively. Under these conditions, pyruvate recycling is easily characterized by the ^{13}C NMR multiplicities of the lactate C3 resonance. (U-$^{13}C_3$) lactate (d_{23}: doublet, δ: 20.9 ppm, 37.0 Hz,) enters the tricarboxylic acid cycle after conversion to (U-$^{13}C_3$) pyruvate and decarboxylation to (1,2-$^{13}C_2$) acetyl-CoA. In the tricarboxylic acid cycle, succinate and its subsequent metabolism leads to generation of approximately equimolar amounts of both (1,2-$^{13}C_2$)- and (3,4-$^{13}C_2$) fumarate, malate and oxaloacetate. If these molecules leave the cycle, via pyruvate recycling, they will generate 50% (1,2-$^{13}C_2$)- pyruvate (d_{12} in lactate: 55 Hz) and 50% (3-^{13}C) pyruvate (s). Additionally, considerable quantities of acetate may be produced, derived from both the original (U-$^{13}C_3$) lactate or from recycled (1,2-$^{13}C_2$)- or (3-^{13}C) pyruvate, originating (1,2-$^{13}C_2$)-, (1-^{13}C)- and (2-^{13}C) acetate, respectively. Thus, it is possible to determine the relative contribution of pyruvate recycling by measuring the relation between the central singlets (s) derived from recycled (3-^{13}C) lactate and (2-^{13}C) acetyl-CoA and the outer doublets (d) derived from the original (U-$^{13}C_3$) lactate (20.9 ppm, d) or unrecycled (1,2-$^{13}C_2$) acetate (24.1 ppm, d). Spectral deconvolution and quantification of relative peak areas were performed with NUTS (Acorn, Freemont, CA, USA).

Results and Discussion. Figure 1A shows a representative spectrum obtained from the medium of C6 cells incubated with (U-$^{13}C_3$) lactate for 48h. Note the significant contributions of central singlets derived from recycled (2-^{13}C) acetate or (3-^{13}C) lactate as compared to the outer doublets derived from (1,2-$^{13}C_2$) acetate or (U-$^{13}C_3$) lactate. This finding reveals unambiguously the presence of pyruvate recycling in these tumour cells. In particular, a relative contribution of recycled vs total pyruvate of 2.47±0.01% can be calculated in this case (48 hr). Figure 1B shows the time course of pyruvate recycling in the medium of C6 cells incubated with (U-$^{13}C_3$) lactate (filled bars) or (2-^{13}C) pyruvate (empty bars). The relative contribution of pyruvate recycling is larger with pyruvate (10.53±0.95% for 48 hr) than with lactate as a substrate. This result demonstrates the redox sensitivity of this pathway, revealing increased activity under the more oxidized NADP/NADPH redox states, such as those obtained with pyruvate. This is consistent with the recycling being determined by malic enzyme activity, a process sensitive to the NADP/NADPH redox state, activated under oxidized redox. In summary, our results demonstrate the presence of significant contributions of pyruvate recycling in tumor cells and reveal an important redox sensitivity for this process.

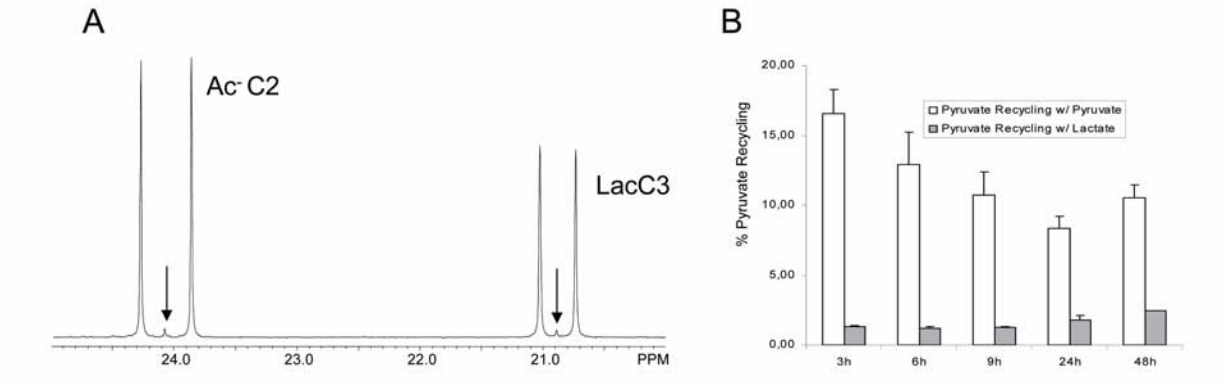

Fig.1: Pyruvate recyling in C6 glioma cells incubated with (U-$^{13}C_3$) lactate and (2-^{13}C) pyruvate. **A.** Representative ^{13}C NMR spectrum of lactate C3 and acetate C2 resonances from the medium of cells incubated with (U-$^{13}C_3$) lactate for 48 hours. Note the existence of singlets revealing recycling in both resonances (arrows). **B.** Time course of pyruvate recycling in the medium of C6 cells incubated with (U-$^{13}C_3$) lactate (filled bars) and (2-^{13}C) pyruvate (empty bars). Values are shown as the percentage of recycled pyruvate in relation to the total pyruvate.

References. (1) Cerdán, S., Künnecke, B., and Seelig, J. 1990. Cerebral metabolism of [1,2-$^{13}C_2$] acetate as detected by *in vivo* and *in vitro* ^{13}C NMR. *J. Biol. Chem.*, 365: 12916-26. **(2)** Waagepeterson, H.S., Qu, H., Hertz, L., Sonnewald, U., and Schousboe, A. 2002. *Neurochem. Res.*, 27: 1431-7. **(3)** Rodrigues, T.B., and Cerdán, S. 2005. *MRM*, 54: 1014-19.