

# Examination of Mouse Mammary Tumor Cell Metabolism with Hyperpolarized [ $^{13}\text{C}$ ] Pyruvate in the Presence and Absence of Glutamine

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**Introduction:** Glutamine is a critical metabolite for the rapid growth of many cell types including stimulated lymphocytes and many cancers. In addition to being a source of nitrogen, it provides cellular energy, reducing equivalents for biosynthesis (NADPH) and TCA cycle anaplerosis (1). Therapeutic reduction of its metabolism in cancer cells may be a useful approach for slowing tumor growth. In this work, we examined the metabolism of hyperpolarized [ $^{13}\text{C}$ ]pyruvate (HP pyruvate) in mouse mammary tumor cells in complete cell culture medium. Subsequently, metabolism was examined in the absence of all glutamine. We anticipated several possible outcomes could occur: (a) the lack of amine groups from glutamine would result in reduced conversion of pyruvate to alanine (b) the loss of energy production from glutamine would be compensated by an increase in the lactate formation rate and (c) the loss of anaplerotic flux from glutamine would be compensated by an increased flux of pyruvate to oxaloacetate through pyruvate carboxylase.

**Methods:** Mouse mammary tumor cells (EMT6/SF) were grown in DMEM medium that contained 10% dialyzed fetal bovine serum, 25 mM glucose, 4 mM glutamine, 25 mM HEPES and penicillin/streptomycin (2). For NMR experiments, they were cultured on non-porous micro-carriers (Pronectin-F, Solohill, Ann Arbor, MI) with a cell perfusion system described previously (2). The microcarriers were perfused in a tightly-packed bed, inside a sealed 20-mm glass NMR tube.  $^{13}\text{C}$  and  $^{31}\text{P}$  spectra were obtained with a 9.4 Tesla 89-mm bore NMR spectrometer and a direct-detection multi-nuclear probe (Varian Inc., Palo Alto, CA) (2). [ $^{13}\text{C}$ ]Pyruvic acid was hyperpolarized with a 3.35 T Hypersense system (Oxford Instruments, Oxfordshire, UK). The OX63 radical (Oxford) was used for polarization and following approximately 90 minutes of microwave radiation, the sample was heated with water, mixed rapidly with a physiologic buffer at  $\sim 37^\circ\text{C}$  and injected into the cell perfusion system.  $^{13}\text{C}$  spectra were acquired with 10 degree pulse and a 6 second interpulse delay. The degree of polarization was  $\sim 10\%$  and the initial  $^{13}\text{C}$  signal to noise (SNR) following injection was between 1000 and 3000:1. During the injection of pyruvate, the standard perfusate medium was not used and the volumetric flow rate of the injected buffer was approximately equal to the perfusate rate (12 ml/min).

**Results:** During growth at standard glucose and glutamine levels and prior to hyperpolarization experiments,  $^{31}\text{P}$  NMR spectroscopy (for NTP measurements (3)) indicated that the viable cell number was approximately  $5 \times 10^8$ . With injection of 15 mM HP pyruvate in the presence of glucose and glutamine, the primary metabolite observed was [ $^{13}\text{C}$ ]lactate (see below). Carbon dioxide and bicarbonate were not detected, even though we have observed that EMT6/SF cells consume high levels of oxygen in this system (2). Surprisingly, a small peak with a maximum SNR of 5:1 appeared at 34.5 ppm; the time course for this resonance was similar to that of lactate. (This peak was not present when HP pyruvate was examined in a 20-mm NMR tube without cells.) [ $^{13}\text{C}$ ]Alanine may have been present at 177 ppm. The other resonances could not be definitively identified. The relatively large resonance at 175.8 ppm was not present in the original HP [ $^{13}\text{C}$ ]pyruvate. The perfusate was changed to medium without glutamine but with 15 mM pyruvate and 1 mM  $\text{NH}_4\text{Cl}$  for approximate 1.5 hours. Subsequently, it was changed to medium without pyruvate for 15 minutes to wash the culture. Injection of 15 mM HP pyruvate + 1 mM  $\text{NH}_4\text{Cl}$  resulted in much slower lactate production than was observed with glutamine in the medium. The resonance at 34.5 ppm was not affected by the absence of glutamine but no alanine was observed. No resonances consistent with pyruvate carboxylase flux such as oxaloacetate, aspartate or malate were observed.

**Discussion:** Many of the findings of this study were unexpected. For example, in studies with  $^{13}\text{C}$  glucose, EMT6/SF cells normally produce significant amounts of labeled alanine. The ratio of labeled lactate to labeled alanine is generally 4:1 (2). In this study, that ratio was approximately than 20:1. Another interesting finding is that lactate formation was reduced when pyruvate plus  $\text{NH}_4\text{Cl}$  was substituted for glutamine. Therefore, the reduced energy production caused by omitting glutamine does not appear to be compensated for with increased flux through lactate dehydrogenase (and presumably glycolysis). Lastly, no metabolites were observed that would suggest pyruvate carboxylase is active. In a few cancer cell lines, we have observed that under some conditions, this enzyme is active. Thus, the primary effect of omitting glutamine from the medium would appear to be an overall reduction in metabolic activity. This would be desirable as a therapeutic approach if an agent were found that could reduce the flux of glutamine to the TCA cycle.

**Conclusions:** We have demonstrated that in a system of cultured cells, hyperpolarized [ $^{13}\text{C}$ ]pyruvate can be used effectively as a metabolic tracer with exceptionally high signal to noise. A single culture can be used repeatedly for a time series of studies, provided that good sterile technique is maintained. Elimination of glutamine from the medium caused a readily detectable reduction in lactate formation that could have interesting implications for therapies that inhibit consumption of this metabolite.

## References:

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