**Effect of PKM2 Activator and 2-Deoxyglucose Treatments on Cancer Metabolism Measured in vivo by hyperpolarized 13C MR Spectroscopic Imaging**

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**Target Audience** Researchers and clinicians who are interested in anti-cancer treatment strategy.

**Purpose** Metabolic reprogramming of tumor-specific metabolism to normal state has been tried as an alternative therapeutic strategy for anti-cancer treatment. For instance, elevated glucose metabolism and lactate (Lac) fermentation (Warburg effect), one of the most well-known metabolic phenotypes of tumor metabolism, has been received much attention. A recent study showed that small molecule activators of PKM2, TEPP46, inhibit xenograft tumor growth1. PKM2, the M2 isoform of pyruvate (Pyr) kinase, plays a role in the last step of glycolysis, converting phosphoenolpyruvate (PEP) to Pyr. So far, PKM2 is expressed in all tested cancer cells1. PKM2 exists either as a dimer or a more active, tetrameric form. The less active dimer is thought to favor rapid cell division by the diversion of glycolytic intermediates into biosynthetic pathways2. More recently, Tee et al. hypothesized that the use of a glucose analogue, 2-deoxyglucose (2DG), in combination with the PKM2 activator will accelerate the uptake of the toxic 2DG in tumors3, and showed that the combination therapy was indeed more effective than single treatment of TEPP46 or 2DG in breast cancer cells. Another study4 of PKM2 treatment on lung cancer cells showed a metabolic difference in treated cells relative to vehicle-treated controls using hyperpolarized 13C MR spectroscopy, which enables a real-time investigation of metabolic kinetics, suggesting that 13C-labeled Lac-to-Pyr ratio might be a biomarker to measure the treatment effect. In this study, we applied the PKM2-2DG treatment to tumor-bearing mice and observed the therapeutic response in Pyr metabolism using hyperpolarized 13C Pyr MR spectroscopic imaging (MRSI).

**Methods** Approximately 5x10^6 H1299 lung cancer cells were injected into mice subcutaneously 8-12 weeks before the imaging study. The progression of the tumors was volumetrically monitored daily to achieve comparable tumor sizes (~500 mm^3). All mice (n=9) were imaged with hyperpolarized 13C MRSI in three groups: 2DG- (n=2, 60 mg/kg body weight), TEPP46- (n=3, 1 mg/kg body weight), and combination-treated (n=4). Animals were anesthetized with 1-1.5 % isoflurane in oxygen (~1.5 L/min) and a catheterized in a tail vein before being placed at the center of a clinical GE 3T MR scanner and a high performance insert gradient coil (G_{max}=500 mT/m, slew rate_{max}=1865 mT/m/ms). A custom-made 13C-1H dual-channel coil was used for both RF excitation and signal acquisition. Vital signs were monitored throughout the experiments, and the body temperature was maintained ~36.5 °C using a temperature-regulated air heater. The homogeneity of the B0 field over the tumor was manually optimized with a point-resolved spectroscopy sequence using the linear shim currents. Single-shot spiral chemical shift imaging (CSI) with spectral undersampling (field of view = 43.5x43.5 mm^2, 6mm slice thickness, matrix size = 16x16, variable flip angle RF excitation, temporal resolution = 3s, 16 time points, 32 echoes, spectral bandwidth = 280 Hz) was used for dynamic metabolic imaging of hyperpolarized [1-13C]Pyr and its products. The 13C spectra were acquired fs after the start of a bolus injection (300 μL of 80mM Pyr over ~20s) at baseline and 2 hr after the treatments. For anatomical references, both T2-weighted fast spin-echo and contrast-enhanced T1-weighted spin-echo images were acquired. Integrated signal for individual metabolite peak in time-averaged spectrum was used to calculate Lac-to-Pyr ratios. Paired student t-test was performed to evaluate statistical significance of treatment effects.

**Results and Discussion** Lac/Pyr ratios in tumors had a large variation between animals probably due to the discrepancy in blood vessel development and perfusion. Nonetheless, Lac/Pyr consistently increased in all mice when they were treated with TEPP46 and 2DG (0.27±0.12 at baseline to 0.43±0.09 at 2hr post-treatment, P<0.05), whereas no significant change in the ratio was observed neither 2DG-only (P>0.2) nor PKM2-only (P>0.2) treated groups. The increase in the ratio suggests that there might be a synergic anti-cancer mechanism of the PKM2 activator and 2DG. The interpretation for Lac/Pyr increase in the dual-treated group could be controversial, but it is possibly because tumors look for any energy source while upregulated 2DG uptake in the tumor cuts off glucose availability as an inhibitory substitute. Therefore, the injected [1-13C]Pyr bolus could be used as a primary source in the tumor, leading to more Lac production.

**Conclusion** The TEPP46-2DG combination therapy showed metabolic perturbation in tumors in vivo, suggesting a potential therapeutic value of the treatment strategy. Lac/Pyr ratio in tumors after an injection 13C-Pyr might serve as a precursor to assess the treatment effect, or possibly a measure for the degree of glucose starvation in tumors.


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