BIOPSY-BASED 1-H AND 31-P NMR SHOWS REGIONAL METABOLIC HETEROGENEITY WITHIN CANINE SPONTANEOUS TUMORS

S. Kraft[1], S. Lana[1], H. Tuttle[1], L. Ireland[1], J. Brown[2], and N. Serkova[2]

[1]Colorado State University, Fort Collins, CO, United States, [2]University of Colorado Health Sciences Center, CO, United States

Introduction: Aggressive or malignant tumor behavior has been associated with higher phosphomonoester / phosphodiester (PME/PDE) ratios and high phosphocholine (PCho) from increased membrane synthesis and tumor proliferation [1]. On the other hand, increased lactate is associated with increased aerobic glycolysis, hypoxia and necrosis [2]. It has also been shown *in vitro*, that acidic microenvironment increases cell death which is associated with increased levels of PDE, especially glycerophosphocholine (GPC) [3]. Biopsy-based high-resolution NMR spectroscopy should allow sampling of submacroscopic tumor regions to map metabolites geographically. Thus far, biopsy-based metabolic profiling by NMR of naturally-occurring cancers has been limited, with very little focus on geographic variation [4]. Metabolic mapping should give us insight into the patterns and regions of most active growth and acidosis within tumors, and provide increased accuracy regarding tumor behavior over histological tumor grading.[3]

Methods: Tumor samples were collected *in vivo* from 11 anesthetized canine patients with a variety of spontaneous malignant tumors (4 lymphosarcomas, 4 soft tissue sarcomas, 2 carcinomas, 1 malignant mast cell tumor). Using a punch biopsy, each peripheral sample was obtained within the first 0.5cm deep to the pseudocapsule of the tumor. The central sample was collected from the central tissue region also by punch biopsy. Samples were immediately snap-frozen in liquid nitrogen and processed for NMR using dual methanol/chloroform extraction to obtain water-soluble and lipid-soluble fractions. ***Proton quantitative NMR*** was done with a 500 MHz high-resolution Bruker DRX system and an inverse TXI 5mm- or 1mm(micro)- probes. Trimethylsilyl propionic-2,2,3,3-d4 acid was used an external standard for chemical shift reference and for metabolite quantification. ^{1}H peaks were integrated using 1D-WINNMR program for metabolite concentrations. ***Phosphorous quantitative NMR*** was done on a Bruker 300 MHz Avance spectrometer equipped with a 5-mm QNP 31P/13C/19F/1H probe, using a composite pulse decoupling (CPD) program. Methyl diphosphoric acid (MDP, 2.3 mmol/l D2O) was used as an external standard.

Results: Spontaneous canine tumors displayed similar metabolic features as human cancers. Typical phosphorous and proton spectra comparing the center and periphery of the same tumor are shown in Figures 1 and 2, respectively. There were consistent differences in PME/PDE ratios, as well as in lactate and glutamate concentrations between tumor centers and peripheries despite intertumoral variation in absolute concentrations. PME/PDE ratios of tumor peripheries were significantly higher than from the tumor centers ($p<0.05$) and averaged 266%. Lactate values of peripheral samples averaged 55% of the central tumor lactate values (Figures 3 and 4). Increased concentration of lactate in the tumor cores (acidic milieu) correlated well with increased concentrations of GPC. ($r=0.89$)

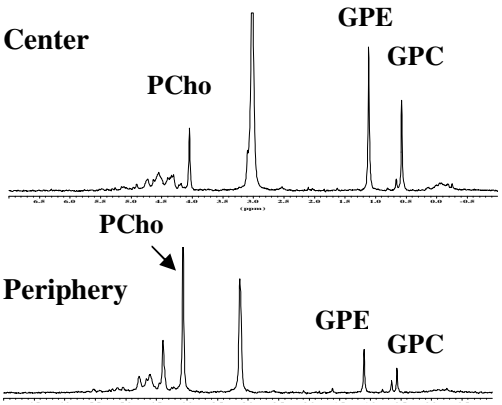


Figure 1. Typical 31-P spectra showing higher PME in tumor periphery (BOTTOM) versus tumor center (TOP).

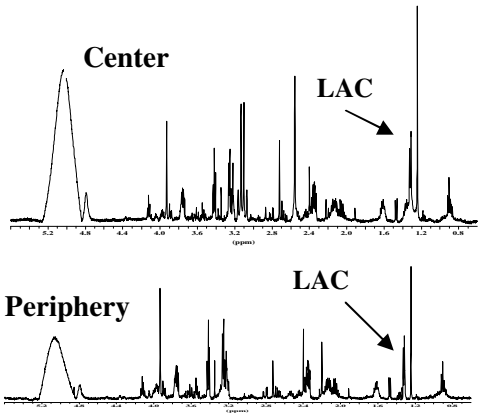


Figure 2. Typical 1-H spectra showing higher lactate tumor center (TOP versus tumor periphery (BOTTOM).

Discussion: Macroscopic tumor heterogeneity has been well documented but the corresponding metabolic variations within a tumor are challenging to study in naturally-occurring tumors. *In vivo* MR spectroscopy lacks the spatial resolution to assess small geographic metabolic variations, whereas this study shows that NMR of tumor biopsies allowed resolution of smaller differences while sampling *in vivo*. Dogs with naturally-occurring tumors were an excellent translational model because canine tumor metabolism and physiology proved to be similar to human tumors.

Conclusion: This study demonstrated the value of biopsy-based NMR for mapping of tumor metabolites geographically. Even within this variety of malignant tumor types, phosphomonoesters were consistently increased in tumor peripheries, and lactate/glutamate/GPC were elevated centrally.

Figure 3. Average Metabolite Concentrations

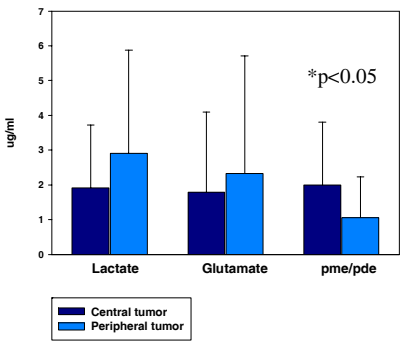
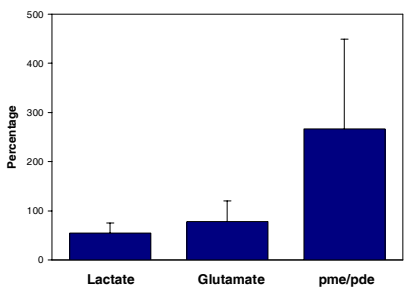


Figure 4. % Metabolite in Periphery/Center



References:

1. **Glunde and Serkova, Pharmacogenomics, 2006**
2. **Griffin and Shockcor, Nat Rev Cancer 2004**
3. **Galons et al., Magn Reson Med, 1995**
4. **Cheng LL et al, Neuro-Oncology 2000**