Relationship between Oxygenation Status and Tumor Metabolites. Noninvasive Evidence for Aerobic Glycolysis (Warburg Effect) in Tumor by Sequential EPR Oxymetric Imaging and MRS

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Summary Tumor hypoxia is an important prognostic factor in treatment outcome in radiotherapy. Cancer cells harbored within hypoxic regions of solid tumors exhibit resistance to radiation therapy and some kinds of chemotherapy, therefore contribute to treatment failure. Non-invasive methods to assess tumor hypoxia such as Electron Paramagnetic Resonance Imaging (EPRI) can provide useful *a priori* information to guide therapy. Lactate is a one of the main metabolic peaks of Magnetic Resonance Spectroscopy (MRS) and its imaging (MRSI) i.e. Chemical Shift Imaging (CSI) detectable in tumor region. The lactate is generally the end product of the non-oxidative glycolysis, and has been reported to correlate with tumor hypoxia. However, one of the most common phenotype of human cancer cell lines is aerobic glycolysis (Warburg Effect) where tumor cells obtain as much as 50 % of their energy (ATP) by metabolizing glucose to lactate even in the presence of oxygen suggesting that lactate production in tumor occurs in aerobic tumors as well as hypoxic tumors. To investigate the dependence between tumor oxygen status and lactate and/or other MRS detectable peaks, we developed a sequential EPR based 3D oxygen imaging technique followed by MRI and MRS. The pulse EPR imager was designed to operate at 300 MHz which enables to be combined with 7T MRI for anatomy and MRS using a common RF coil without disturbing the object. This combined system of EPRI oximetry and 7T MRS clearly shown the oxygenation dependent MRS metabolic change in tumor.

Method The 3D oxygen imaging in Squamous cell carcinoma (SCC) bearing mouse was performed using a homebuilt 300 MHz pulsed EPR imager with single point imaging (SPI) sequence. After EPR imaging, the mouse was transferred to 7T MRI scanner with the common RF coil. Anatomical images were obtained by fast spin echo sequence, and then MRS was performed using PRESS sequence. The 2.5×2.5×2.5 mm^3 voxel was selected for MRS based on the oxygen maps by EPRI. Blood volume images were calculated from images pre and post ultrasmall superparamagnetic iron oxide (USPIO) injection.

Results Oxygen maps from EPR imaging studies show that tumor oxygen distribution was heterogeneous with both hypoxic and relatively well oxygenated regions. MRS spectra obtained from these regions show that a strong lactate peak was detected in even well oxygenated region, suggesting aerobic glycolysis processes in this tumor.

However, a relatively small lactate peak in hypoxic region may be explained by limited blood supply *i.e.* limited glucose supply as evidenced from the blood volume images. Total choline peak also positively correlated with tumor pO_2. Choline peak has been reported to correlate with cell proliferation level, and tumor growth rate is slower in hypoxic condition. Therefore, positive relationship between choline content and oxygenation level can be ascribed to difference in the tumor proliferation rate dependent on the oxygenation status. These data suggest that EPR oxygen imaging combined with MRS provides unique opportunity for better understanding of the relationship between MRS metabolic peaks and tumor oxygen status.