Choline Production and Her-2/neu Expression in Breast Cancer measured by MRI/MRSI

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Introduction. Many *in vivo* and *in vitro* experiments have demonstrated that elevated concentrations of choline-containing metabolites are associated with malignant transformation and may also reflect important characteristics of the tumor microenvironment such as oxygenation and pH[1]. The amplification and overexpression of Her-2/*neu* has been linked to poor prognosis in several human cancers such as breast, prostate, lung, ovary, and colon cancers. We previously observed increased phosphocholine in breast epithelial cells transfected with the *erbB2* (Her2neu) oncogene[2]. In this study, we performed experiments to simultaneously assess the distribution of Her-2/neu receptors and total choline in human breast cancer xenograft model using noninvasive MRI and MRSI.

Materials and methods. Her-2/neu expressing human breast cancer BT-474 tumors were grown in female athymic mice supplemented with an Estradiol slow-release pellet. For MR imaging of Her-2/neu receptors a two-step targeted approach that includes biotinylated trastuzumab and an avidinGdDTPA conjugate was implemented. Biotinylated Herceptin was injected I.V. at a dose of 1mg and was followed after 48h with 6mg of the avidinGdDTPA I.V. Quantitative T_1 MR images were obtained by a saturation-recovery multi-slice spin-echo pulse sequence on a 9.4T Bruker Biospec spectrometer at 24h and 48h post contrast administration. Tumor regions with high expression of the Her-2/neu receptors are characterized by a fast T_1 relaxation rate due to the specific binding of the contrast agent to the antibody-prelabeled Her-2/neu receptors[3]. Following T1 measurement studies 2D proton spectroscopic imaging (TR/TE = 1500/40 ms) was performed using spin-echo pulse sequence with VAPOR water suppression. CSI images were recorded from a 4 mm single slice through the center of the tumor with in-plane resolution of 1 mm. As the animal was not removed from the magnet between the studies both T1 and CSI maps are spatially coregistered. Experiments were repeated for a group of 5 animals. All image processing was performed with a dedicated software developed on an IDL platform.

Results and discussion. Choline maps were reconstructed from 2D CSI data by integrating the total choline peak at 3.2 ppm shown in Fig. 1 in all image pixels. Typically, highly non-uniform distribution of choline was detected in all BT-474 xenografts.

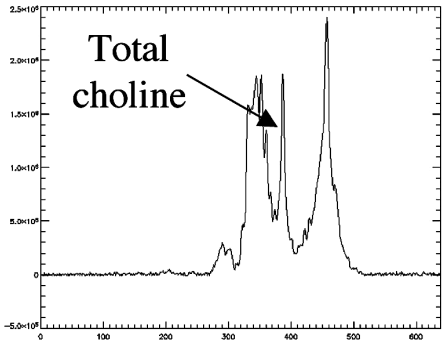

Figure 1. Representative spectrum of elevated total choline within the tumor demonstrates a detectable choline signal.

Her-2/neu overexpressing BT-474 breast tumor mouse xenograft also demonstrated nonuniform uptake and binding of the Her-2/neu targeted MR contrast agent as shown in a quantitative R1 map in Fig. 2. As also shown in this figure there is a coarse association of the tumor regions with high choline with areas of signal enhancement in Her-2/neu targeted imaging (Fig. 2B). Due to the highly inhomogeneous distribution of choline in the tumor it is difficult to define quantitative correlation function between the images. however a simple image correlation analysis for this particular tumor gives Manders overlap coefficient of 0.65 calculated with JACoP plugin for NIH ImageJ image processing software.

A. **B.**

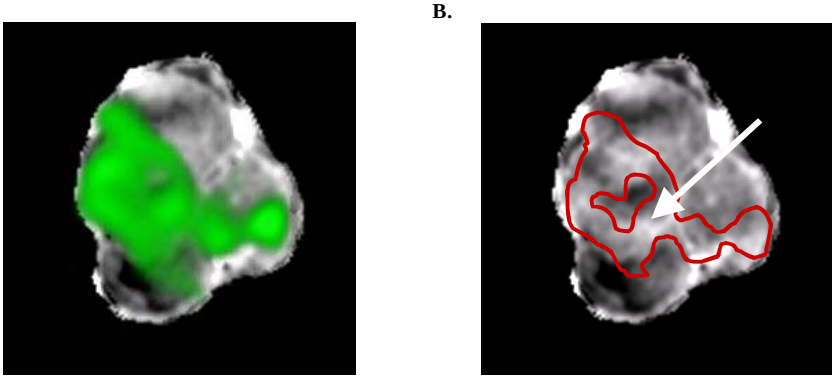

Figure 2. **A**: Combined image of a central slice from R1 ($T1^{-1}$) map of a BT-474 mouse tumor xenograft (grayscale) and a corresponding choline map reconstructed from 2D CSI data set (green). **B**: Association between tumor areas with high Her-2/neu expression with choline distribution outlined by red contours and indicated by an arrow.

Conclusion

Preliminary data demonstrate coarse association between total choline distribution and MR detectable Her-2/neu labeling in BT-474 tumor model that suggests that these tumor features may correspond to more aggressive tumor regions.

References. [1] Glunde, K., C. Jie, et al. (2004). "Molecular causes of the aberrant choline phospholipid metabolism in breast cancer." Cancer Res **64**(12): 4270-6. [2] Aboagye, E. O. and Z. M. Bhujwalla (1999). "Malignant transformation alters membrane choline phospholipid metabolism of human mammary epithelial cells." Cancer Res **59**(1): 80-4.[3] Artemov, D., N. Mori, et al. (2003). "Magnetic resonance molecular imaging of the HER-2/neu receptor." Cancer Res **63**(11): 2723-7.

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