Mining Lipid Signatures in a Breast Tumor Model by Combining Magnetic Resonance Spectroscopic Imaging and Mass Spectrometric Imaging

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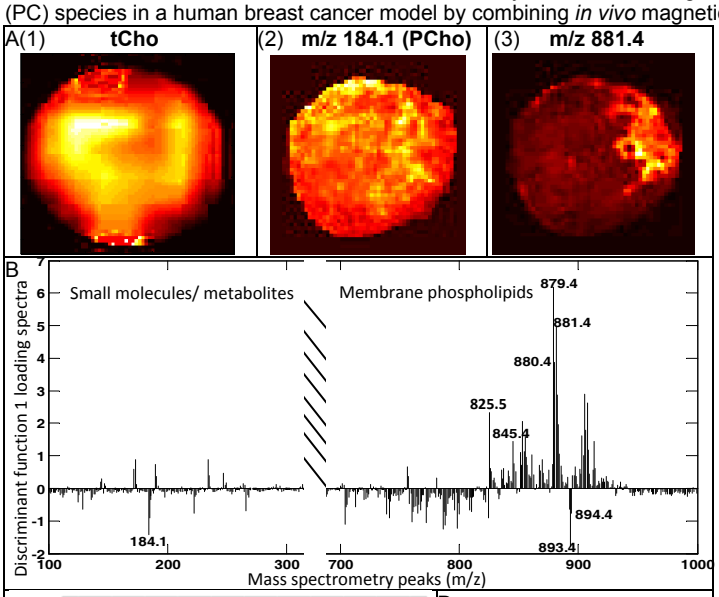
Introduction: The intensity of the total choline (tCho) signal in magnetic resonance spectroscopic imaging (MRSI) of tumors is spatially heterogeneous. Magnetic resonance spectroscopy (MRS) studies have shown an elevation of phosphocholine (PCho) and total choline-containing metabolites (tCho) in breast cancer cells and tumors [1]. However, *in vivo* H^1 MRSI at high spatial resolution with the spectral resolution to resolve PCho, glycerophosphocholine (GPC), and free choline (Cho), which are the three overlapping signals that the tCho signal consists of, is currently unavailable. Mass spectrometry imaging (MSI) of histologic tumor sections is able to detect the spatial distribution of these choline metabolites and thousands of other molecules from the tissue surface. In this study, we have investigated the correlations between tCho, lipid metabolites, and phosphatidylcholine (PC) species in a human breast cancer model by combining *in vivo* magnetic resonance imaging (MRI) and MRSI with *ex vivo* MSI.



M/Z	COMMON_NAME
184.1	Phosphocholine (PCho) ($C_5H_{15}PNO_4$) [M+H]+
881.4	PC(22:4/22:6) [M+H]+
893.4	PC(22:0/22:4) [M+H]+
825.5	PC(18:4/22:6) [M+H]+
845.4	PC(14:0/26:0) [M+H]+
894.5	PC(20:3/22:6) [M+K]+
880.4	PC(20:0/20:2) [M+K]+

Figure A (1): MRSI tCho map (3.2 ppm) of breast tumor xenograft; and corresponding MALDI MSI map of m/z 184.1 (Figure A (2)) and m/z 881.4 (Figure A (3)). B: Discriminant function 1 loading spectra of 3392 randomly selected high-tCho-containing and low-tCho-containing voxels. C: Scattering plot of discriminant function 1 scores and function 2 scores obtained in the classification of high- and low-tCho-containing voxels. D: Candidate MALDI MSI peaks obtained by our data analysis.

Methods: Human MDA-MB-231-HRE-tdTomato breast cancer cells were orthotopically grown in nude mice. Both 3-dimensional (3D) water-unsuppressed H^1 MRSI to detect water, and water-suppressed 3D MRSI to detect metabolites, was performed on tumors *in vivo*. Inherently registered 3D T1-weighted images were acquired to measure the tumor anatomic structure as a reference for MRSI. Each tumor was cryo-sectioned into fiducially marked 10-µm thick slices to perform MSI. Matrix-assisted laser desorption ionization (MALDI) MSI was performed on a Q-TOF instrument (Synapt, Waters) as described previously [2, 3] to detect small molecules as well as lipids. We used our co-registration platform based on Ponceau S fiducial markers and shape characteristics that allowed us to fuse MRI, MRSI, MSI images [4]. The 3D tCho volume was segmented by considering the voxels above 10% of the area under the histogram as high-tCho-containing area. The remaining voxels under 10% were considered as the low-tCho-containing area. The corresponding 3D MALDI high-tCho-containing and 3D MALDI low-tCho-containing voxels were pooled, from which 1545 high-tCho-containing voxels and 1847 low-tCho-containing voxels were selected randomly, followed by principal component analysis (PCA) to reduce the dimension and noise of these MALDI MSI data. 80% variance of the PCA projection data was analyzed by Fisher linear discriminant analysis (FDA) to classify high-tCho-containing voxels and low-tCho-containing voxels through these MALDI data. The m/z range between m/z 100 and m/z 1000 of MALDI MSI data was analyzed and the loading spectra of discriminant function 1 were sorted to select candidate m/z peaks, which mostly contributed to the differentiation between high- and low-tCho-containing voxels. The molecular m/z peak candidates were identified by searching an online lipidomics database (http://www.lipidmaps.org/) with the resulting m/z values as keywords.

Results: tCho (3.2 ppm) was observed by *in vivo* MRSI (Fig. A (1)). Several molecules such as the phosphocholine molecular ion PCho (m/z 184.1) as well as membrane phospholipid species such as m/z 893.4 and m/z 881.4 displayed the highest peaks in the discriminant function 1 loading spectrum obtained from MALDI MSI data. Fig. A (1) shows a representative MRSI tCho map. Fig. A (2) and (3) show the corresponding MALDI MSI distribution of PCho (m/z 184.1) and PC (22:4/22:6) (m/z 881.4). Fig. B shows the discriminant function 1 loading spectrum of 3392 randomly selected high- and low-tCho labeled voxels in MALDI MSI. Fig. C shows the scattering plot of FDA discriminant function 1 and function 2 scores obtained in the classification of high-tCho-containing and low-tCho-containing voxels. Fig. D lists candidate molecular m/z peaks obtained by our classification analysis.

Discussion and Conclusions: The PCho peak at m/z 184.1 was identified in our previous MALDI MSI study [5]. The fact that m/z 184.1 also showed a high rank in the present study validates our data analysis method as being useful for identifying molecular peaks in MALDI MSI data that were classified as high- and low-tCho-containing voxels based on the corresponding registered MRSI data of the same tumor. The molecular ions at m/z 881.4, 893.4, 825.5, 845.4, 894.5, and 880.4 were identified as membrane phosphatidylcholine lipid species that negatively correlated with both the tCho map in MRSI and the PCho map of m/z 184.1 in MALDI MSI. This finding suggests that high tCho and PCho regions in breast tumors may be partially caused by increased breakdown and/or reduced biosynthesis of the specific phosphatidylcholines listed in Fig. D. All identified phosphatidylcholine species are currently undergoing further validation by ion fragmentation studies using MSI-based MS/MS methods. By combining MRSI with MALDI MSI, followed by registration and data analysis based on tCho-voxel classification, PCA, and FDA, we identified for the first time some of the specific phosphatidylcholine species that are either broken down in breast tumor regions that contain high tCho, or that experience reduced biosynthesis in these high tCho regions.

References: [1]. Glunde et al. (2011), NMR Biomed 24(6):673-90; [2]. Luxembourg et al. (2004), Anal Chem 76: 5339-44; [3]. Luxembourg et al. (2006), Rapid Commun Mass Spectrom 20: 3435-42; [4]. Jiang et al. (2011), Abstract #: 959, ISMRM, Montreal, CA; [5]. Amstalden van Hove et al. (2010), Cancer Research 70(22): 9012-21. **Acknowledgements:** This work was supported by NIH R01 CA134695.