Biomarkers of Aggressive Breast Cancer Revealed by Combining Magnetic Resonance Spectroscopic Imaging and Mass Spectrometric Imaging

Lu Jiang[1], Kamila Chughtai[2], Tiffany Greenwood[1], Zaver M. Bhujwalla[1], Venu Raman[1], Gert Eijkel[2], Ron Heeren[2], and Kristine Glunde[1]

[1]*Department of Radiology, Johns Hopkins University School of Medic, BALTIMORE, MD, United States,* [2]*FOM-Institute AMOLF, Amsterdam, Netherlands*

Target Audience: Scientists interested in breast cancer imaging and biomarker research.

Introduction: An aberrant choline metabolism is a metabolic hallmark of cancer associated with oncogenesis and tumor progression. Breast tumor regions containing an elevated total choline (tCho) signal as detected with magnetic resonance spectroscopic imaging (MRSI) *in vivo* are likely more aggressive [1] and hypoxic [2]. To discern underlying molecular pathways leading to regions of high tCho in breast tumor, we combined *in vivo* MRSI with matrix-assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI) of aggressive breast tumor. This approach has enabled us to identify proteins that are elevated in regions of high tCho in an aggressive triple-negative breast tumor model.

Methods: 3D MRSI of orthotopic MDA-MB-231-HRE-tdTomato breast tumor xenografts was performed *in vivo* to detect tCho [3]. Tumors were cryo-sectioned throughout, followed by on-tissue tryptic digestion and α-cyano-4-hydroxycinnamic-acid matrix deposition, to perform MALDI-MSI on a MALDI Q-TOF instrument (Synapt, Waters) and detect tryptic peptides. We fused MRSI and MSI in 3D [4]. The 3D MRSI tCho volume was segmented as high-tCho-containing and low-tCho-containing areas. Corresponding MALDI data were analyzed by least absolute shrinkage and selection operator (LASSO) to classify high- and low-tCho-containing voxels. Candidate m/z peaks, which mostly contributed to the differentiation, were obtained from the parsimonious sets of features for discriminating between high-tCho and low-tCho generated from LASSO and identified through an in-house built accurate mass and time (AMT) tag peptide/protein database.

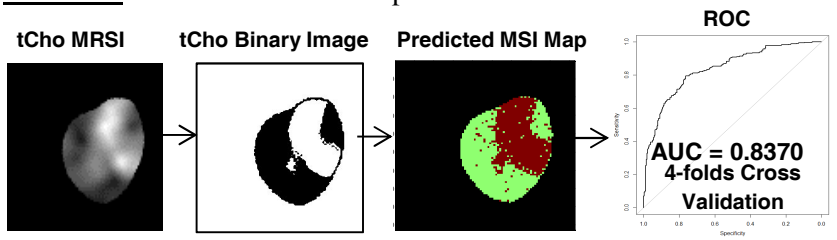

Figure 1: tCho MRSI image, segmented tCho binary image, predicted MSI map, and ROC analysis.

m/z	Protein ID	Description
1019.5	S10AB_HUMAN	Protein S100-A11
1332.6	ALDOA_HUMAN	Fructose-bisphosphate aldolase A
1534.7	TYRO_HUMAN	Tyrosinase
1550.7	HNRPM_HUMAN	Heterogeneous nuclear ribonucleoprotein M
1574.7	SRSF6_HUMAN	Serine/arginine-rich splicing factor 6
1670.8	CY1_HUMAN	Cytochrome c1, heme protein, mitochondrial
1875.9	CLIC1_HUMAN	Chloride intracellular channel protein 1
1896.9	RAN_HUMAN	GTP-binding nuclear protein Ran
2010	K1C18_HUMAN	Keratin, type I cytoskeletal 18
2028	HNRH2_HUMAN	Heterogeneous nuclear ribonucleoprotein H2
2055	GUAA_HUMAN	GMP synthase [glutamine-hydrolyzing]
2221.1	VIME_HUMAN	Vimentin
2242.1	DHE3_HUMAN	Glutamate dehydrogenase 1, mitochondrial
2666.3	PLEC_HUMAN	Plectin
2678.3	CLCA_HUMAN	Clathrin light chain A
2773.3	TYB10_HUMAN	Thymosin beta-10
2852.4	TCEA1_HUMAN	Transcription elongation factor A protein 1
2884.4	G6PI_HUMAN	Glucose-6-phosphate isomerase
2892.4	LDHA_HUMAN	L-lactate dehydrogenase A chain

Table 1: M/z value of tryptic peptide, protein ID, and description of identified proteins.

Results and Discussion: The 3D tCho distribution detected by *in vivo* MRSI (3.2 ppm) was heterogeneous. A tCho binary image was obtained after image segmentation from four tumors. 4-folds cross validation was applied to all the voxels within the tumors, an AUC of 0.8370 under the ROC curve was obtained (Figure 1). Around 20 candidate tryptic peptides were obtained by LASSO classification of high- *versus* low-tCho-containing voxels from a total of four tumors (Table 1), such as for example, Fructose-bisphosphate aldolase A; (ALDOA, m/z 1332.6), Glutamate dehydrogenase 1 (m/z 2242.1), L-lactate dehydrogenase A chain (LDHA, m/z 2892.4), Cytochrome c1 heme protein (CY1, m/z 1670.8), and Glucose-6-phosphate isomerase (G6P1, m/z 2884.4). All other identified tryptic peptides are undergoing further validation by ion fragmentation studies using MS/MS methods. Fructose-bisphosphate aldolase A (ALDOA, m/z 1332.6) plays a key role in glycolysis and gluconeogenesis. Phospholipase D2 (PLD2) directly interacts with ALDOA via its PH domain [5]. PLD2 is phosphatidylcholine (PC)-specific phospholipases D, which catalyzes the hydrolysis of PC to produce phosphatidic acid and choline. This may explain why ALDOA is highly associated with regions of high tCho. The association of other candidate proteins and tCho is currently undergoing further investigation in our lab. By combining MRSI with tryptic on-tissue digestion MALDI-MSI, followed by registration and tCho-voxel classification, we identified for the first time some specific proteins that are differentially expressed in breast tumor regions that contain high tCho.

References: [1]. Glunde K. et al. Nat Rev Cancer. 11(12):835-48; [2]. Glunde K. et al. Cancer Res. 68(1):172-80; [3]. Jiang L. et al. NMR Biomed. 26(3):285-98; [4]. Jiang L. et al. Neoplasia. 14(8):732-41. [5] Kim JH. et al. Biochemistry. 41(10):3414-21. This work was supported by NIH R01 CA134695.