Choline Metabolite Ratios from NMR as Markers of Human Breast Cancer

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Introduction: Breast cancer is the most common malignancy affecting women in the United States, causing more than 40,000 deaths each year. Contrast-enhanced breast MRI provides increased sensitivity for detecting cancer as compared to conventional imaging. Although this increased sensitivity is important, there is a need to improve the specificity of MRI. Magnetic resonance spectroscopy (MRS) at high field strength (4T and greater) has increased the specificity of MRI through the ability to detect and measure distinct molecular constituents of breast tissue (1). In particular, *in vivo* MRS can detect a signal assigned to total choline (tCho), which arises from a variety of choline-containing metabolites. These metabolites are key compounds in the metabolism of phosphatidylcholine (PtdCho), a major component of the cell membrane (2). The tCho signal is typically elevated in breast cancer compared to normal breast tissue. What is not known is the extent to which each individual PtdCho metabolite [choline (Cho), phosphocholine (pc), and glycerophosphocholine (gpc)] contributes to the increased tCho peak *in vivo*. Here we report *in vitro* ^{1}H NMR spectroscopy results on fine-needle aspirate (FNA) biopsies of lesions from breast-cancer patients, several of whom were also studied by *in vivo* MRS.

Methods: Along with standard biopsy, FNAs were acquired from suspicious lesions in 7 subjects using a 22-gauge needle. Several of these subjects had previously been scanned using localized ^{1}H MRS (TE, 23 ms; TR, 2.0 s) on a Varian 4-T MRI scanner. All of the lesions studied here were confirmed as breast cancer by histopathologic analysis of the standard biopsy. The FNA samples (typically 2-3 per lesion) were immediately placed into a single cryogenic vial containing 250 µL of phosphate-buffered saline in D_2O (d-PBS, pH 7.3) and frozen in dry ice. The sample was then stored in a -80°C freezer until immediately before NMR analysis. After thawing, 5 µL of 100 mM 2,2,3,3-d_4 sodium 3-trimethylsilylpropionate was added as a chemical shift reference, the solution transferred to a 5-mm, restricted-volume, susceptibility-matched NMR tube (Shigemi), and d-PBS added to a total volume of 300 µL. High resolution ^{1}H NMR spectra were acquired with water presaturation on a Varian Inova 400-MHz (9.4 T) spectrometer in a manner similar to that of Mountford and coworkers (3,4). Scan conditions were: 90° pulse, 6.2 µs; TR, 4.7 s; spectral width, 6 kHz in 32k complex points; 256 transients. Peak assignments, which are consistent with the literature, were confirmed by spiking with pure standard compounds. The spectra were analyzed individually off-line using NUTS (Acorn NMR). Resonances were fit for gpc, pc, Cho, the combined peak from creatine and phosphocreatine [(P)Cr] at 3.04 ppm, the interference taurine (Tau), and any resolved but unidentified interferences, yielding peak area ratios for the Cho-containing metabolites relative to (P)Cr (Table).

Table. Metabolite Ratios in Breast Cancer Subjects

Subject	gpc/ (P)Cr	pc/ (P)Cr	Cho/ (P)Cr	gpc/ pc
1	1.92	3.40	0.55	0.57
2	5.07	9.96	2.38	0.51
3	3.44	4.32	1.11	0.80
4	5.90	5.25	2.08	1.12
5	1.58	1.25	0.62	1.26
6	1.89	11.43	1.64	0.17
7	1.88	12.98	2.37	0.14
Mean	3.10	6.94	1.54	0.65
±s.d.	±1.76	±4.48	±0.79	±0.43

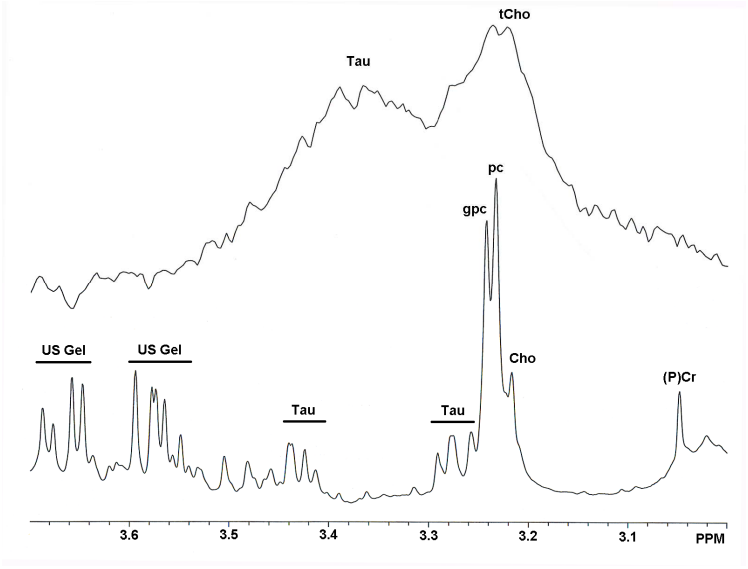

Figure. *In vivo* (top) and *in vitro* (bottom) ^{1}H MR spectra of a breast tumor from the same subject. US Gel, gel used for ultrasound exam. Scan conditions differ *in vivo* vs. *in vitro*.

Results: The Figure shows the pertinent 3.0-3.7-ppm region of *in vivo* and *in vitro* spectra from the same subject. The *in vivo* spectrum was roughly aligned to the *in vitro* spectrum using tCho and pc. In the *in vivo* spectrum, the tCho peak is a single, relatively broad resonance, in this case partially overlapping the nearby resonance arising from Tau and perhaps other metabolites (1). The weak resonance due to (P)Cr is not seen *in vivo*, as it is *in vitro*. Attempts at resolving the component metabolites of the *in vivo* tCho peak by computer resolution enhancement were unsuccessful.

Unlike in previous reports (3,4), gpc, pc, Cho, and Tau are partially resolved here in the *in vitro* spectra of the FNAs. We attribute this to slightly higher magnetic field, susceptibility-matched tubes, lack of a capillary insert, and gradient shimming used here. Restriction of the sample to the active coil volume improves signal-to-noise ratio for these small samples. In the *in vitro* spectrum the Cho-containing metabolites are largely resolved from Tau interference. The Table gives the quantitative results for 7 FNA-biopsied subjects. As expected for these confirmed breast cancer lesions, the gpc/pc ratios are all <≈1.

Discussion: Cellular studies have demonstrated the importance of these three Cho-containing metabolites for studying breast cancer. Bhujwalla and coworkers (5,6) found a gpc-to-pc switch for immortalization of mammary epithelial cells and malignant progression. Typically the gpc/pc ratio progressed from much greater than 1 to much less than 1 as cells progressed from normal to malignant. Moreover, the total amount of Cho-containing metabolites was increased in breast cancer relative to normal cells (7). The increase in total Cho-containing compounds was primarily due to pc, and to a lesser extent gpc. Our results for actual human tumor samples are consistent with the cellular findings. Pc was usually the major metabolite in the *in vivo* tCho signal in cancer, whereas the contribution of gpc varied substantially, and that of Cho was always minor.

High-resolution NMR spectroscopy of breast-lesion FNAs, taken in conjunction with high-field *in vivo* ^{1}H MRS, should elucidate the molecular origins of the increased tCho resonance seen *in vivo*. The gpc/pc ratio may serve as an indicator of degree of malignancy. False positives arising from non-Cho-containing metabolites such as Tau should be readily distinguished by *in vitro* NMR.

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