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 glycero phosphocholine (gpc)] contributes to the increased tCho peak in vivo. Here we report in vitro 1H 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 1H 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 -80°C freezer until immediately before NMR analysis. After thawing, 5 μL of 100 mM 2,2,3,3-d2 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 1H MRS (TE, 23 ms; TR, 2.0 s) on a Varian 400-MHz (9.4 T) spectrometer in a manner similar to that of Mountford and coworkers (3,4). Sample 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).

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 on 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 vivo MRI.


Figure. In vivo (top) and in vitro (bottom) 1H 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.