

^1H NMR analysis of choline metabolites in fine-needle-aspirate biopsies of breast cancer

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Abstract

Object The relative amounts of choline (Cho), phosphocholine (PC), and glycerophosphocholine (GPC) may be sensitive indicators of breast cancer and the degree of malignancy. Here we implement some simple modifications to a previously developed ^1H NMR analysis of fine-needle-aspirate (FNA) biopsies designed to yield sufficient spectral resolution of Cho, PC, and GPC for usable relative quantitation of these metabolites.

Materials and methods FNA biopsies of eighteen breast lesions were examined using our modified procedure for direct ^1H NMR at 400 MHz. Resonances of choline metabolites and potential interferences were fit using the computer program NUTS.

Results Quantitation of PC, GPC, and Cho relative to each other and to (phospho)creatine was obtained for eleven confirmed cases of infiltrating ductal carcinoma. Reliable results could not be obtained for the remaining cases primarily due to interference from lidocaine anesthetic.

Conclusion Some simple modifications of a previously developed ^1H NMR analysis of FNAs yielded sufficient spectral resolution of Cho, PC, and GPC to permit usable relative quantitation at 400 MHz. In 9 of the 11 quantified cases the sum of GPC and Cho exceeded 42 % of the total choline-metabolite peak area.

Keywords Phosphocholine · Glycerophosphocholine · Lidocaine · Biopsy · Phosphoethanolamine

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Abbreviations

| | |
|-----|--------------------------------|
| FNA | Fine needle aspirate |
| PBS | Phosphate buffered saline |
| PC | Phosphocholine |
| GPC | Glycerophosphocholine |
| Cho | Choline |
| PtC | Phosphatidylcholine |
| PL | Phospholipid |
| PE | Phosphoethanolamine |
| GPE | Glycerophosphoethanolamine |
| IDC | Infiltrating ductal carcinoma |
| ILC | Infiltrating lobular carcinoma |

Introduction

One metabolic signature of cancer is its aberrant phosphatidylcholine (PtC) metabolism [1]. Early NMR studies suggested that phospholipid (PL) metabolism was altered in tumors [1]. Under proper conditions, several

compounds directly involved in PtC metabolism can be observed in ^1H NMR spectra, including choline (Cho), phosphocholine (PC), and glycerophosphocholine (GPC). While PC and GPC are commonly referred to as the precursor and degradation product, respectively, of PtC metabolism, both metabolites function in multiple roles in a complex network of metabolic processes [2, 3]. Studies of cultured cellular preparations demonstrated the importance of these three metabolites for studying breast cancer [4–11]. Bhujwala and coworkers [4, 7] found a GPC-to-PC switch for immortalization of mammary epithelial cells and malignant progression. Typically the GPC/PC ratio progressed from >1.0 to <1.0 as cells progressed from normal to malignant. Moreover, the total amount of Cho-metabolites was increased approximately 2–10 times in breast cancer relative to normal cells [4, 6, 9]. These results suggest that the relative amounts of PC, GPC, and Cho may be sensitive indicators of breast cancer and the degree of malignancy.

Several approaches are available for the application of ^1H MR techniques to breast cancer detection and characterization. The most common approach concentrates on in vivo techniques such as single-voxel magnetic resonance spectroscopy (MRS) or magnetic resonance spectroscopic imaging (MRSI) [12–14]. In vivo ^1H MRS is attractive because it is noninvasive and can obtain tumor-localized spectra. Beyond cost its disadvantages are its relatively low sensitivity (tumors must be 1–2 cm in size and usually larger) and its limited information content. Even at 7 T, the *N*-methyl resonances of the choline-containing metabolites are not individually resolved and give rise to a single peak from the total NMR-visible Cho (tCho) in the tumor [12–15]. Moreover, other metabolites may also contribute to the tCho peak. It is generally believed that changes in tCho are dominated by changes in PC [4, 13]. However, measurement of tCho has not been linked directly and unambiguously in the same human subjects to changes in individual Cho-containing metabolites.

Although it is possible to resolve PC and GPC using ^{31}P MRS in vivo [15], these key metabolites cannot be resolved using ^1H MRS in vivo; in the latter case it is necessary to study biopsy samples (or resected tumor). In typical high-resolution ^1H NMR spectra of aqueous extracts, the *N*-methyl resonances of these soluble metabolites can be well resolved, along with numerous other resonances from a wide variety of cellular metabolites [16–18]. This method, particularly at high magnetic fields, can justify a metabonomic approach [17]. However, biopsy specimens suitable for extraction and ^1H NMR in solution are typically 20 mg or more. Sample extraction is labor intensive and risks degradation of metabolites during workup. Once extracted, the sample is no longer suitable for subsequent histological characterization.

To avoid many of the problems with tissue extracts, high-resolution magic-angle-spinning (MAS) ^1H NMR spectroscopy (MAS-NMR) has been applied to intact tissue samples [19–21]. Here an intact tissue sample of about 20 mg is spun at a rate of about 5 kHz at 54.74° relative to the magnetic field to remove line broadening from magnetic susceptibility differences within the sample due to the solid or solid-like components [19]. The result is a ^1H NMR spectrum with a spectral resolution approaching that of the comparable extract [19–21]. After MAS-NMR analysis, the sample is usually available for histological characterization. However, ^1H MAS-NMR requires special equipment, and the technique can be somewhat more difficult to implement than standard high-resolution NMR. Because some small amount of residual broadening usually remains with MAS, it is best applied at higher magnetic fields (≥ 600 MHz for ^1H).

A simple method that surprisingly has not seen wider popularity is the direct high-resolution NMR analysis of fine-needle-aspirate (FNA) biopsies. In this approach, as pioneered by Mountford and coworkers [22–24], an FNA biopsy of the breast (or of a resected tumor) taken with a 22–24-gauge needle is directly analyzed in buffered D_2O using standard ^1H NMR techniques. Direct NMR analysis of FNAs is easy to implement on common NMR spectrometers, requires a small sample and minimal preparation, and minimizes sample losses and degradation. The sample is not suitable for later histologic characterization. Although spectral resolution is improved relative to in vivo MRS, as initially implemented [22–24] at 8.5 T (360 MHz), the *N*-methyl resonances of the three key PtC metabolites are not resolved.

Here we report simple refinements of the FNA ^1H NMR analysis that in combination permit partial resolution and quantitation of the component Cho metabolites. This modified approach has the advantages of minimal biopsy size, speed and ease of sample preparation, as well as resolution of the key PL metabolites at field strengths that are modest for high-resolution NMR. We also investigate potential interferences from both endogenous metabolites and exogenous compounds originating from the clinical breast-cancer examination. Finally, we report results for eleven confirmed cases of infiltrating ductal carcinoma.

Materials and methods

The protocol was approved by the University of Cincinnati Institutional Review Board. Along with standard large-core needle biopsy, FNAs were acquired from suspicious breast lesions of 18 subjects using a 22-gauge needle. Seventeen lesions were confirmed as breast cancer [16 infiltrating ductal carcinoma (IDC), one infiltrating lobular carcinoma

(ILC), one benign] by histopathologic analysis of the standard biopsy tissue specimens. With a few exceptions (see Table 1), the identical procedure was followed for all samples from initial sampling through NMR data acquisition. Three FNA samples per lesion were immediately (within 1 min) combined in a single (except in one case, see Discussion), pre-cooled cryogenic vial containing 250 μL of phosphate-buffered saline in D_2O (d-PBS) at pH 7.3 and frozen in dry ice at -78.5°C . Within several hours, the sample was then stored in a -80°C freezer for, typically, 1 week to 2.5 months until immediately before NMR analysis. After thawing, 2–5 μL of 10 mM 2,2,3,3- d_4 sodium 3-trimethyl-silylpropionate was added as a chemical shift reference at 0.00 ppm, and the solution was transferred to a 5-mm, restricted-volume, susceptibility-matched NMR tube (Shigemi, Allison Park, PA, USA). Additional d-PBS was used to rinse the vial and bring the total NMR sample volume to 270–300 μL , yielding a sample height approximately equal to the RF coil height. Finally, a susceptibility-matched plug was inserted with careful removal of air bubbles. No capillary insert was used, and samples were not spun. Gradient shimming was performed on the D_2O deuterium signal using the routine on the Varian Inova 400-MHz (9.4 T), narrow-bore spectrometer. High-resolution ^1H NMR spectra were acquired at 21°C with water presaturation in a manner similar to that of Mountford [22–24] using a triple-tuned ^1H – ^{13}C – ^{15}N inverse probe. Scan conditions were: 90° pulse, 6.2 μs ; TR, 4.7 s; spectral width, 6 kHz in 8 k complex points; 256 transients. The entire procedure from sample thawing until completion of initial NMR data acquisition for quantitation took 1 h or less for all samples. The FIDs were exponentially multiplied with 0.2 Hz line broadening and Fourier transformed to 16 k complex points.

Peak assignments of the Cho-metabolites and the key endogenous interferences were confirmed by spiking with standard compounds. The presence of the surgical preparations lidocaine hydrochloride 1 %, lidocaine hydrochloride 1 % with epinephrine 1:100,000 (Hospira Inc, Lake Forest, IL, USA), and ultrasound transmission gel (Aquasonic 100, Parker Laboratories, Fairfield, NJ, USA) were also confirmed by spiking. The spectra were analyzed individually off-line using NUTS (Acorn NMR, Palo Alto, CA, USA). Resonances in the region of 3.0–3.5 ppm were fit for the following known compounds: GPC, PC, Cho, phosphocreatine/creatine [(P)Cr], taurine (Tau), *myo*-inositol (Inos), phosphoethanolamine (PE), glycerophosphoethanolamine (GPE), lidocaine, and β -D-glucose. Any resolved but unidentified interferences were also fit when necessary to improve the fit of adjacent known metabolite signals. After performing a polynomial baseline fit of the 3.0–3.5 ppm extract of each Fourier-transformed spectrum, signals were fit to Lorentzian-Gaussian line shapes by

adjusting intensities, linewidths, chemical shifts and the minor Gaussian component as needed to obtain a visual match between the simulated and actual spectrum. Because the weights of the FNA samples were unknown (and problematic to obtain reliably using this sampling method), absolute metabolite concentrations could not be determined. Although here we used a TR of 4.7 s and identical pulse conditions for all samples, a longer TR could readily be used to insure that the metabolite ratios are insensitive to metabolite T_{1s} , which were not determined.

Results

Figure 1 shows the partial 400-MHz ^1H NMR spectrum of an FNA breast-cancer biopsy (#3 in Table 1). Unlike in previous reports [22–24], GPC, PC, Cho, and Tau are now partially resolved *ex vivo*. These assignments are consistent with the literature and were confirmed in actual FNA spectra by spiking with pure standards. Under our standard conditions of temperature and pH, the chemical shifts of the Cho-metabolites were very stable. Spectra sequentially acquired from a single FNA sample at 21, 37 and 21°C showed a significant but reversible increase in linewidths (20–30 % for Cho-metabolite *N*-methyl singlets) at higher temperature. Also shown are the individual peak fits using NUTS. Table 1 gives the ratios GPC/PC and Cho/PC for the 11 samples that yielded quantifiable spectra. Six spectra (5 IDC and one benign) were considered unusable due to severe interference from lidocaine and/or β -D-glucose (see below); no PtC metabolites were detected in the spectrum of the ILC sample.

Figure 2 shows the pertinent region of the spectrum of an FNA sample (#1 in Table 1) that contains the anesthetic lidocaine. The primary quartet of lidocaine, centered at about 3.32 ppm and assigned to the methylene protons on the *N*-diethyl moiety, does not interfere with the Cho-metabolite analysis. A partial spectrum of the lidocaine preparation used here (Fig. 3) displays a minor quartet at about 3.22 ppm, which at high lidocaine concentrations can interfere with the Cho-metabolite analysis. Figure 4 shows the spectrum of an FNA sample with a high relative concentration of lidocaine, as well as a significant level of β -D-glucose. Interference of the lidocaine minor quartet and of the H-2 proton of β -D-glucose with the Cho-metabolite resonances is indicated. The Cho-metabolite ratios could not be determined reliably in this and similar cases.

As given in Table 1, GPC/PC varies from 0.14 to 1.26 (mean 0.70 ± 0.36) and Cho/PC varies from 0.06 to 0.50 (mean 0.23 ± 0.12) for these samples. Neither GPC/PC nor Cho/PC correlated with tumor grade. Also given in Table 1 are ratios of the individual Cho-metabolites to (P)Cr. None of these ratios correlated with tumor grade.

Table 1 Metabolite ratios for breast cancer samples

| Subject | IDC grade | GPC/PC | Cho/PC | GPC/(P)Cr | PC/(P)Cr | Cho/(P)Cr |
|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1 ^a | 1 | 0.57 | 0.16 | 1.92 | 3.40 | 0.55 |
| 2 ^a | 2 | 0.51 | 0.24 | 5.07 | 9.96 | 2.38 |
| 3 | 3 | 0.80 | 0.26 | 3.44 | 4.32 | 1.11 |
| 4 | 2 | 1.26 | 0.50 | 1.58 | 1.25 | 0.62 |
| 5 ^b | 2 | 0.17 | 0.14 | 1.89 | 11.43 | 1.64 |
| 6 | 2 | 1.21 | 0.28 | 3.30 | 2.73 | 0.77 |
| 7 | 3 | 0.14 | 0.18 | 1.88 | 12.98 | 2.37 |
| 8 | 3 | 0.84 | 0.35 | 2.82 | 3.35 | 1.17 |
| 9 | 2 | 0.57 | 0.23 | 2.74 | 4.82 | 1.09 |
| 10 | 2 | 0.79 | 0.11 | 4.71 | 5.98 | 0.66 |
| 11 | 2 | 0.81 | 0.06 | 2.54 | 3.13 | 0.19 |
| Mean \pm SD | 2.18 \pm 0.60 | 0.70 \pm 0.36 | 0.23 \pm 0.12 | 2.90 \pm 1.16 | 5.76 \pm 3.91 | 1.14 \pm 0.72 |

^a Samples stored at -80 °C for about 7 months

^b Sample stored in ice for <1 h before transfer to -80 °C freezer

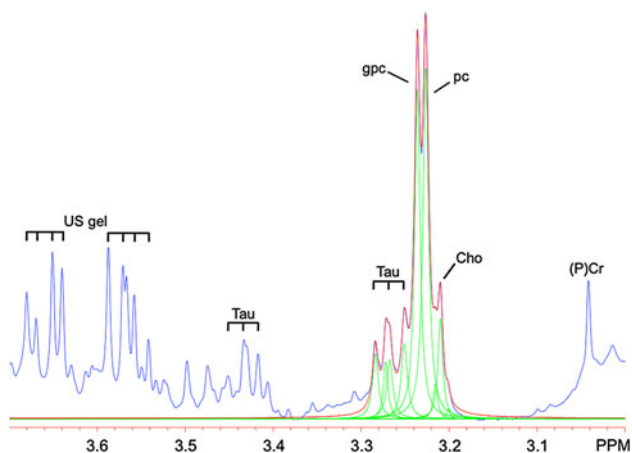


Fig. 1 The 3.0–3.7-ppm region of the ex vivo ^1H NMR spectrum of an FNA biopsy of a human breast tumor (#3 in Table 1). US gel, gel used in ultrasound examination; (P)Cr, (phospho)creatine. Also shown are the individual peak fits using NUTS

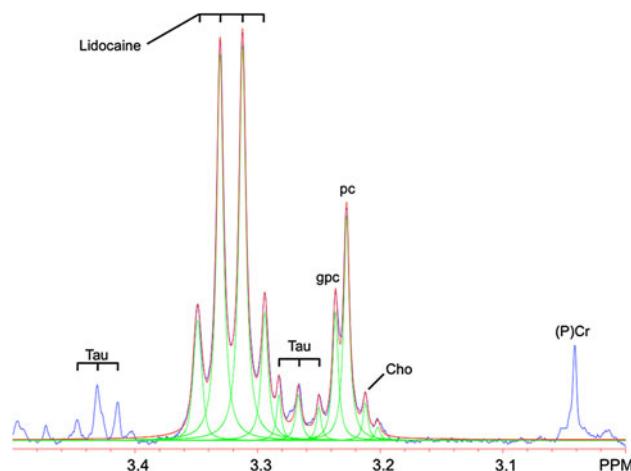


Fig. 2 The 3.0–3.5-ppm region of the ex vivo ^1H NMR spectrum of an FNA biopsy of a human breast tumor containing lidocaine (#1 in Table 1). The individual peak fits using NUTS are shown

Discussion

Modified FNA analysis

Our magnetic field is somewhat higher than used by Mountford and coworkers [22–24]. The Jagannathan group [18] marginally improved on the original analysis using a straightforward approach at 9.4 T with occasional resolution of Cho from GPC/PC, but did not achieve the resolution we report here. Thus the modest increase in magnetic field from 8.5 T [22–24] to 9.4 T appears responsible for part of the resolution improvement we observed. Several additional refinements further improved our spectral resolution. The near-ambient sample temperature of 21 °C yielded narrower linewidths and minimized

sample equilibration time relative to higher temperatures. By permitting completion of the analysis within 1 h after sample thawing, these conditions minimized sample degradation while maintaining sufficient spectral resolution for the analysis. Use of 5-mm, restricted-volume, susceptibility-matched NMR tubes (and plugs) allowed for improved shimming and narrower resonances. Also, we did not spin the samples or use a capillary insert, further simplifying the shimming process. We suspect that sample spinning can degrade resolution due to motion of the FNA-biopsy particulates, although we did not investigate this possibility in detail. Restriction of the sample to the active coil volume improved signal-to-noise ratio for these small samples. Moreover, in this approach the entire sample was analyzed and losses due to leaching into wash or storage buffers, as

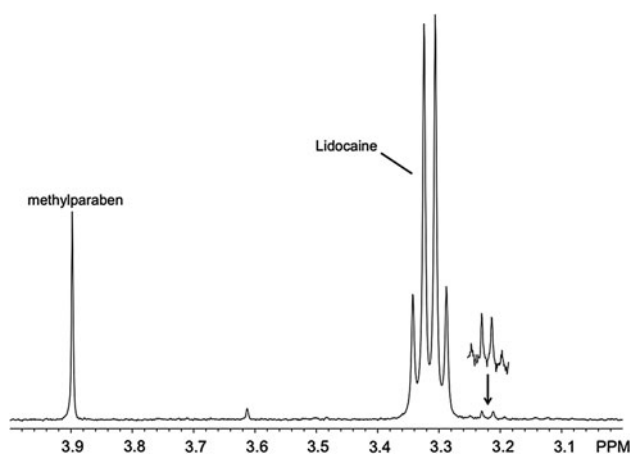


Fig. 3 The 3.0–4.0-ppm region of the ^1H NMR spectrum of the lidocaine-based anesthetic used during breast biopsy. The *insert* shows the minor component at about 3.22 ppm

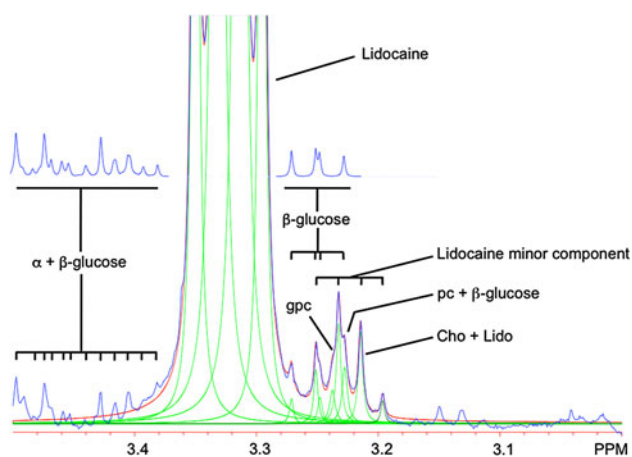


Fig. 4 The 3.0–3.5-ppm region of the ex vivo ^1H NMR spectrum of an FNA biopsy of a human breast tumor containing significant β -D-glucose and excessive lidocaine. Lidocaine-minor-component and β -D-glucose interferences are indicated

may be the case for in vitro or MAS-NMR of biopsies, were avoided [25].

In view of prior work [4–11], our focus has been on resolving the Cho-containing metabolites and determining intensity ratios within that group. An alternative approach to quantitation is to use the resonance at 3.04 ppm due to (P)Cr as an internal standard. This is the approach taken by Mountford and coworkers in their original work [22]. While the (P)Cr resonance often serves as an internal standard for in vivo studies of brain, it is not usually observed in vivo for breast. Perhaps unlike for brain tissue, in vivo (P)Cr (PCr + Cr) is probably not a good reference for ex vivo analysis of breast tissue, although PCr degradation should not have a major effect on its intensity as PCr degrades to Cr. Nevertheless, we included the results relative to (P)Cr in Table 1 to allow comparison to previous

work, while our focus overwhelmingly remains on the ratios of the Cho-metabolites.

One concern is metabolite degradation during the approximately 1-h period of sample workup and spectral acquisition at 21 °C. Previous work [reviewed in [26]] suggests that the potentially labile metabolites GPC and PC are sufficiently stable under the conditions of our analysis for reliable relative quantitation. In some cases samples were rerun over several hours, up to 1 day, in order to perform repeated spiking experiments to confirm peak identification. Significant changes in Cho-metabolite ratios were observed in only one sample over this time course. We plan to investigate using a lower temperature for sample preparation and spectral acquisition with the goal of further minimizing the possibility of sample degradation during the 1-h period after thawing.

Endogenous interferences

We consider a number of common metabolites that resonate in close proximity to the Cho-metabolite resonances and may interfere with the FNA analysis. Many of these have been identified in better-resolved extract spectra at 600 MHz [16]. The component farthest upfield (~ 3.25 ppm at 400 MHz) of the upfield multiplet of Tau partially overlaps with GPC, but its effect is easily accommodated by curve fitting. Glycerophosphoethanolamine (GPE) has a multiplet with components ranging from 3.28 to 3.31 ppm, which may obscure the Tau multiplet but should not interfere with the Cho-metabolite analysis. This is also the case for *myo*-inositol and *scyllo*-inositol, with resonances at about 3.29 ppm or higher. A possible ethanolamine multiplet at about 3.15 ppm poses no problem of interference. Although breast adipose tissue can make a major contribution to ex vivo ^1H MR spectra and in vivo, the closest lipid resonance to the N-methyl grouping is from diallylic methylene protons at 2.77 ppm [27].

Phosphoethanolamine (PE) displays a multiplet centered at ~ 3.24 ppm in our analysis. For breast cancer, PE has been shown to be the major component of the phospho-monoester (PE + PC) resonance group in ^31P MRS [28]. In ^1H NMR, interference from PE will be reduced by a factor of 9–18 relative to the N-methyl resonances of the three Cho-metabolites for equal concentrations. We did not detect PE in any of our samples.

Of more concern is the H-2 proton of β -D-glucose, which displays a multiplet centered at about 3.25 ppm. The component of this multiplet farthest upfield occurs at the same chemical shift as PC in our analysis. Its presence is indicated by the α - and (partially suppressed) β -D-glucose H-1 doublets at 5.24 and 4.65 ppm, respectively, as well as numerous additional signals between 3 and 4 ppm, including the upfield β -D-glucose H-2 multiplet. Figure 4 shows a spectrum with a significant and unambiguous

interference from β -D-glucose (in addition to lidocaine, see below). The ratio of β -D-glucose to Cho-metabolites was significant for our analysis in 5 of the 18 cases studied. We made no attempt to correct for this interference. A dramatic difference in β -D-glucose content was observed between two samples from a single patient. One sample with significant blood contamination had substantially more β -D-glucose interference than the other, uncontaminated sample. While tissue heterogeneity cannot be ruled out as a source of the β -D-glucose concentration variation in this case, the potential for introducing interferences such as β -D-glucose and non-tumor-derived Cho-metabolites via blood during biopsy suggests that minimization of blood contamination during FNA sampling is desirable.

Exogenous interferences

To our knowledge, exogenous NMR interferences have not been reported for breast biopsies. Van Asten et al. [29] have reported detection of the echo gel used for ultrasound (US) guidance of prostate needle biopsies, while Santos et al. [30] reported detection of local anesthetic (a benzocaine-containing product) in transrectal-US-guided biopsies of prostate. Lidocaine, used as an anesthetic prior to endoscopy, has been detected in NMR spectra of healthy gastric mucosa [31].

In this work we occasionally detected resonances from the US gel typically used prior to biopsy. Figure 1 shows an NMR spectrum of an FNA containing residual US gel. Resonances from the US gel do not interfere with the Cho-metabolite analysis, although they would complicate a more comprehensive analysis of the spectrum. In practice, careful cleansing of the skin surface subsequent to the US exam and prior to biopsy should minimize this contaminant.

Figure 2 shows the spectrum of an FNA containing a typical residual amount of the anesthetic lidocaine. The major resonances of the anesthetic preparation used here (lidocaine-HCl, with the preservative methylparaben) do not interfere with the Cho-metabolite analysis. Unfortunately, minor resonances, which may arise from the free-base form of lidocaine, can interfere at high lidocaine concentrations. Figure 3 is the spectrum of lidocaine in d-PBS buffer with a vertical expansion showing a quartet centered at about 3.22 ppm arising from the minor component at a level of about 1.7 % of the major component. Depending on the relative ratio of residual anesthetic to Cho-metabolites in the FNA sample, the lidocaine interference could be severe, as it was for 4 of the 18 samples studied here. Figure 4 shows the spectrum of an FNA sample with a very high lidocaine concentration. Significant lidocaine interference is suggested by the presence of a resonance at about 3.195 ppm due to the component of

the minor quartet that is farthest upfield, although an unidentified, endogenous metabolite also appears at this chemical shift. In Fig. 4, the three peaks of interest are contaminated by the two largest peaks of the quartet. We have found that the potential for lidocaine interference varies greatly, with some FNA spectra showing no lidocaine, while others are dominated by the anesthetic. One solution may be to switch to an alternative anesthetic. Polocaine, for which the closest resonance is at about 3.12 ppm, should not interfere. However, chlorprocaine, another alternative anesthetic, will interfere with the analysis in the same manner as lidocaine.

Application to cancer diagnosis

The Cho-metabolite ratios in Table 1 are largely consistent with the diagnosis of breast cancer based on previous cellular and biopsy studies [4, 5]. Interestingly, the PC resonance truly dominates for only two of the samples (#s 5 and 7) in Table 1. For the other nine samples, the sum of GPC and Cho constitutes either a majority or a substantial fraction of the total intensity that is normally attributed to the tCho resonance in vivo. Interestingly, Moestue et al. [32] found that the profile of Cho-metabolites varies with molecular subtype. In basal-like tumors, GPC concentrations were higher than those for PC, whereas the reverse was true for luminal-like tumors [32].

We are currently using the technique to study FNA biopsy samples from patients with a variety of breast lesions. One goal of our work is to link changes in the tCho resonance seen in vivo with changes in the ratios of the various Cho-metabolites determined by ex vivo ^1H NMR analysis of FNA biopsies on the same subjects [33].

Conclusions

We have implemented some simple modifications of a previously developed ^1H NMR analysis of FNAs that yield sufficient spectral resolution of Cho, PC, and GPC to permit usable relative quantitation at 400 MHz and 21 °C with minimal analysis time and without sample extraction or MAS. Potential interferences from exogenous compounds such as the anesthetic lidocaine have been characterized. Endogenous interferences, which may also contribute to the tCho resonance observed using in vivo ^1H MRS, have also been characterized. Results for 11 confirmed cases of infiltrating ductal carcinoma were successfully obtained. In most cases, the sum of GPC and Cho constitutes either a majority or a substantial fraction of the total intensity that is normally attributed to PC.

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References

- Podo F (1999) Tumour phospholipid metabolism. *NMR Biomed* 12:413–439
- Podo F, Canevari S, Canese R, Pisanu ME, Ricci A, Iorio E (2011) MR evaluation of response to targeted treatment in cancer cells. *NMR Biomed* 24:648–672
- Glunde K, Bhujwala ZM, Ronen SM (2011) Choline metabolism in malignant transformation. *Nat Rev Cancer* 11:835–848
- Aboagye EO, Bhujwala ZM (1999) Malignant transformation alters membrane choline phospholipid metabolism of human mammary epithelial cells. *Cancer Res* 59:80–84
- Sitter B, Lundgren S, Bathen TF, Halgunset J, Fjosne HE, Gribbestad IS (2006) Comparison of HR MAS MR spectroscopic profiles of breast cancer tissue with clinical parameters. *NMR Biomed* 19:30–40
- Katz-Brull R, Seger D, Rivenson-Segal D, Rushkin E, Degani H (2002) Metabolic markers of breast cancer: enhanced choline metabolism and reduced choline-ether-phospholipid synthesis. *Cancer Res* 62:1966–1970
- Glunde K, Jie C, Bhujwala ZM (2004) Molecular causes of the aberrant choline phospholipid metabolism in breast cancer. *Cancer Res* 64:4270–4276
- Glunde K, Ackerstaff E, Mori N, Jacobs MA, Bhujwala ZM (2006) Choline phospholipid metabolism in cancer: consequences for molecular pharmaceutical interventions. *Mol Pharmaceutics* 3:496–506
- Morse DL, Carroll D, Day S, Gray H, Sadarangani P, Murthi S, Job C, Baggert B, Raghunand N, Gillies RJ (2009) Characterization of breast cancers and therapy response by MRS and quantitative gene expression profiling in the choline pathway. *NMR Biomed* 22:114–127
- Podo F, Sardaneli F, Iorio E, Canese R, Carpinelli G, Fausto A, Canevari S (2007) Abnormal choline phospholipid metabolism in breast and ovary cancer: molecular bases for noninvasive imaging approaches. *Curr Med Imaging Rev* 3:123–137
- Eliyahu G, Kreizman T, Degani H (2007) Phosphocholine as a biomarker of breast cancer: molecular and biochemical studies. *Int J Cancer* 120:1721–1730
- Mountford C, Lean C, Malycha P, Russell P (2006) Proton spectroscopy provides accurate pathology on biopsy and in vivo. *J Magn Reson Imaging* 24:459–477
- Haddadin IS, McIntosh A, Meisamy S, Corum C, Styczynski Snyder AL, Powell NJ, Nelson MT, Yee D, Garwood M, Bolan PJ (2009) Metabolite quantification and high-field MRS in breast cancer. *NMR Biomed* 22:65–76
- Sharma U, Jagannathan NR (2009) Biochemical characterization of breast tumors by in vivo and in vitro magnetic resonance spectroscopy (MRS). *Biophys Rev* 1:21–26
- Klomp DWJ, van de Bank BL, Raaijmakers A, Korteweg MA, Possanzini C, Boer VO, van de Berg CAT, van de Bosch MAAJ, Luijten PR (2011) ^{31}P MRSI and ^1H MRS at 7 T: initial results in human breast cancer. *NMR Biomed* 24:1337–1342
- Gribbestad IS, Sitter B, Lundgren S, Krane J, Axelson D (1999) Metabolite composition in breast tumors examined by proton nuclear magnetic resonance spectroscopy. *Anticancer Res* 19:1737–1746
- Whitehead TL, Kieber-Emmons T (2005) Applying in vitro NMR spectroscopy and ^1H NMR metabonomics to breast cancer characterization and detection. *Prog NMR Spectrosc* 47:165–174
- Kumar M, Seenu V, Julka PK, Srivastava A, Kapila K, Rath GK, Jagannathan NR (2005) Proton MR spectroscopy of human breast cancer. In: Jagannathan NR (ed) *Recent advances in mr imaging and spectroscopy*. Jaypee Bros, New Delhi, pp 312–344
- Sitter B, Bathen TF, Tessem M-B, Gribbestad IS (2009) High-resolution magic angle spinning (HR-MAS) MR spectroscopy in metabolic characterization of human cancer. *Prog NMR Spectrosc* 54:239–254
- Beckonert O, Coen M, Keun HC, Wang Y, Ebbels TMD, Holmes E, Lindon JC, Nicholson JK (2010) High-resolution magic-angle-spinning NMR spectroscopy for metabolic profiling of intact tissues. *Nature Prot* 5:1019–1032
- Sitter B, Bathen TF, Singstad TE, Fjosne HE, Lundgren S, Halgunset J, Gribbestad IS (2010) Quantification of metabolites in breast cancer patients with different clinical prognosis using HR MAS MR spectroscopy. *NMR Biomed* 23:424–431
- Mackinnon WB, Barry PA, Malycha PL, Gillett DJ, Russell P, Lean CL, Doran ST, Barraclough BH, Bilous M, Mountford CE (1997) Fine-needle biopsy specimens of benign breast lesions distinguished from invasive cancer ex vivo with proton MR spectroscopy. *Radiology* 204:661–666
- Mountford CE, Somorjai RL, Malycha P, Gluch L, Lean C, Russell P, Barraclough B, Gillett D, Himmelreich U, Dolenko B, Nikulin AE, Smith ICP (2001) Diagnosis and prognosis of breast cancer by magnetic resonance spectroscopy of fine-needle aspirates analysed using a statistical classification strategy. *Br J Surg* 88:1234–1240
- Lean C, Doran S, Somorjai RL, Malycha P, Clarke D, Himmelreich U, Bourne R, Dolenko R, Nikulin AE, Mountford C (2004) Determination of grade and receptor status from the primary breast lesion by magnetic resonance spectroscopy. *Technol Cancer Res Treat* 3:551–556
- Bourne R, Dzendrowskyj T, Mountford C (2003) Leakage of metabolites from tissue biopsies can result in large errors in quantitation by MRS. *NMR Biomed* 16:96–101
- Komoroski RA, Pearce JM, Mraz RE (2008) ^{31}P NMR spectroscopy of phospholipid metabolites in postmortem schizophrenic brain. *Magn Reson Med* 59:469–474
- Dimitrov IE, Douglas D, Ren J, Smith NB, Webb AG, Sherry AD, Malloy CR (2012) In vivo determination of human breast fat composition by ^1H magnetic resonance spectroscopy at 7 T. *Magn Reson Med* 67:20–26
- Smith TAD, Glaholm J, Leach MO, Machin L, Collins DJ, Payne GS, McCready VR (1991) A comparison of in vivo and in vitro ^{31}P NMR spectra from human breast tumours: variations in phospholipid metabolism. *Br J Cancer* 63:514–516
- van Asten JJA, Cuijpers V, Hulsbergen-van de Kaa C, Soede-Huijbregts C, Witjes JA, Verhofstad A, Heerschap A (2008) High resolution magic angle spinning NMR spectroscopy for metabolic assessment of cancer presence and Gleason score in human prostate needle biopsies. *Magn Reson Mater Phy* 21:435–442
- Santos CF, Kurhanewicz J, Tabatabai ZL, Simko JP, Keshari KR, Gbegenon A, Delos Santos R, Federman S, Shinohara K, Carroll PR, Haqq CM, Swanson MG (2010) Metabolic, pathologic, and genetic analysis of prostate tissues: quantitative evaluation of histopathologic and mRNA integrity after HR-MAS spectroscopy. *NMR Biomed* 23:391–398
- Schenetti L, Mucci A, Parenti F, Cagnoli R, Righi V, Tosi MR, Tugnoli V (2006) HR-MAS NMR spectroscopy in the characterization of human tissues: application to healthy gastric mucosa. *Concepts Magn Reson* 28A:430–443
- Moestue SA, Borgan E, Huuse EM, Lindholm EM, Sitter B, Børresen-Dale A-L, Engebraaten O, Mælandsmo GM, Gribbestad IS (2010) Distinct choline metabolic profiles are associated with differences in gene expression for basal-like and luminal-like breast cancer xenograft models. *BMC Cancer* 10:433–445
- Mahoney MC, Lee JH, Chu WJ, Pearce JM, Cecil KM, Strakowski NM, Komoroski RA (2010) Choline metabolite ratios from MRS as markers of human breast cancer. In: *Proceedings of the 18th scientific meeting, International Society for Magnetic Resonance in Medicine, Stockholm*, p 2765