Review
Imaging biochemistry: applications to breast cancer
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Abstract

The use of magnetic resonance spectroscopy (MRS) to investigate breast tumour biochemistry *in vivo* is reviewed. To this end, results obtained both from patients *in vivo* and from tumour extracts and model systems are discussed. An association has been observed between transformation and an increase in phosphomonoesters (PMEs) detected in the ^{31}P MRS spectrum, as well as an increase in choline-containing metabolites detected in the ^{1}H spectrum. A decrease in PME content after treatment is associated with response to treatment as assessed by tumour volume. Experiments in model systems aimed at understanding the underlying biochemical processes are presented, as well as data indicating the usefulness of MRS in monitoring the uptake and metabolism of some chemotherapeutic agents.

Keywords: choline, magnetic resonance spectroscopy (MRS), ^{31}P MRS, ^{1}H MRS, phosphomonoesters

Introduction

The purpose of this section is to review the potential applications of magnetic resonance spectroscopy (MRS) to non-invasive probing of the underlying biochemistry of cells comprising a breast tumour. MRS does not generate an image of the tumour directly, but the spectroscopic data can now be obtained from a well localised area. Thus the biochemical information obtained from MRS can be interpreted in relation to a defined anatomical location, and images of metabolite distributions can be generated. In using MRS, the first aim is to identify surrogate biochemical markers of cellular transformation, thus differentiating benign tumours from malignant or potentially identifying the different tumour types. Subsequently, prognostic and diagnostic information is sought from the spectrum of malignant tumours. In prognosis, by yielding biochemical information on the tumour composition (eg the presence of a hypoxic or a drug-resistant fraction), MRS could allow the selection of an appropriate treatment. In diagnosis, early detection of

tumour response to treatment, or monitoring of drug uptake and metabolism, could enable rapid optimisation of treatment if the tumour failed to respond. Overall, treatment that is better adapted to the individual patient could result from a better understanding of the biochemical events occurring within the tumour.

So far, most patient and model system studies have focused on ^{31}P MRS. Within the ^{31}P spectrum, the three nucleoside triphosphate (NTP) peaks, composed primarily of ATP, together with the inorganic phosphate (P_i) and phosphocreatine peaks, are indicative of energetic status. The P_i peak can also serve as a measure of intracellular pH. The phosphomonoester (PME) signal, which can be resolved into phosphocholine (PC) and phosphoethanolamine (PE) at higher field strengths or by decoupling, together with the phosphodiester (PDE) peak, composed of glycerol phosphocholine (GPC) and glycerol phosphoethanolamine, are indicative of lipid metabolism.

5-FU = 5-fluorouracil; GPC = glycerol phosphocholine; MR = magnetic resonance; MRS = magnetic resonance spectroscopy; NTP = nucleoside triphosphate; PC = phosphocholine; PDE = phosphodiester; PE = phosphoethanolamine; P_i = inorganic phosphate; PME = phosphomonoester.

Finally, NAD(H) and uridine diphosphate sugars can also sometimes be resolved in the ^{31}P spectrum (Fig. 1).

In spite of its higher sensitivity compared to ^{31}P MRS, ^{1}H MRS has been used less frequently owing to the large signals from water and lipids that often dominate the spectrum, as well as the significant number of metabolite signals distributed over a relatively small chemical shift range. Nevertheless, when water-suppressed spectra are recorded, total choline, total creatine, lipids, glutamate, glutamine, inositols and lactate can be detected, potentially providing diverse biochemical information.

Other nuclei of interest include ^{19}F and ^{13}C. ^{19}F has been used primarily in studies of drug metabolism, particularly 5-fluorouracil (5-FU). The use of ^{13}C MRS in breast cancer has been restricted to model system investigations in which studies of the metabolism of specific pre-labelled compounds such as glucose or choline can be used to elucidate specific alterations in tumour biochemistry.

Considering spectra obtained from patients, these are often of poor sensitivity and resolution owing to technical challenges, the limited sensitivity of the method and low metabolite concentrations. Consequently, the number of *in vivo* patient MRS studies is relatively limited, particularly in breast tissue. To identify the potential uses of MRS in the imaging of biochemistry *in vivo*, results obtained at higher field strengths from breast cancer tumour extracts will be discussed. Data obtained from model systems composed of homogenous cell populations or implanted tumour xenografts will also be presented, concentrating wherever possible on human breast cancer models.

Studies of tumour extracts, cells and xenograft models
Use of MRS to differentiate between benign and malignant tumours
In an effort to differentiate between benign and malignant tumours, early work concentrated on the ^{31}P MRS spectrum of human breast tumour extracts [1•,2]. These studies demonstrated higher levels of PMEs and NTPs in the carcinomas relative to benign tumours. Others have identified an association between the PC component of the PMEs and tumour grade as well as the proliferating fraction [3,4]. The importance of the PME region in identifying cellular transformation was further recognised by pattern recognition studies of spectra obtained from xenografts and compared with normal tissues such as liver, brain or muscle [5]. More recent studies comparing a normal human mammary epithelial cell line and a transformed human breast cancer cell line demonstrated that the levels of PMEs as well as PDEs were extremely low in the normal cells, and significantly less than in the breast cancer cell line [4]. A further assessment of PC as well as total choline content in a series of breast cancer cell lines demonstrated that levels

Figure 1
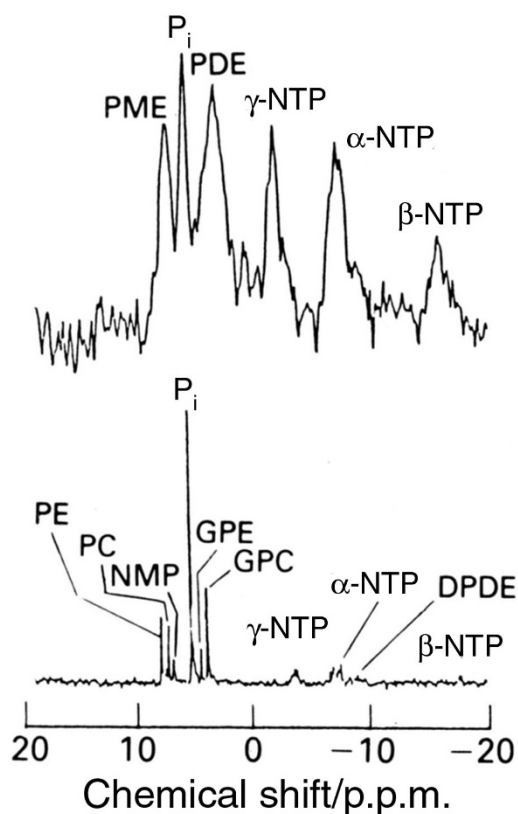
^{31}P MRS spectra of human breast cancer tumour recorded *in vivo* at 1.5 T (top) and recorded *in vitro* after extraction at 5.8 T (bottom) [28]. DPDE, diphosphodiesters; GPE, glycerol phosphoethanolamine; NMP = nucleoside monophosphates. Reprinted from [28] by permission of the publisher, Churchill Livingstone.

of choline-containing metabolites are correlated with malignant transformation and progression [6] as well as the acquisition of a metastatic potential [7].

Such studies have recently been complemented by ^{1}H MRS investigations of extracts from non-involved breast tissue and breast tumours. The spectra demonstrated a high choline content as well as low levels of glucose and increased levels of lactate in the tumour spectra [8•]. Finally, spectra from about 200 fine-needle aspirate samples have shown that the choline peak in the ^{1}H spectrum could be used to distinguish between benign and malignant tumours with relatively high sensitivity and specificity [9].

Use of MRS in understanding tumour biochemistry
As recently reviewed [10•], it is still not entirely clear which are the metabolic changes that lead to the increased levels of PMEs observed in breast and other cancers. Changes have been hypothesised to be associated with an enhanced cell membrane synthesis, cellular

growth, or nutrient availability, as well as with cell signalling by lipid hydrolysis. Nevertheless, some understanding of the biochemical processes involved has been obtained by ^{13}C and ^{2}H MRS, in combination with labelled metabolites such as choline [4,11]. In breast cancer cells, rapid choline transport into the cells has been demonstrated, probably explaining the high levels of PC present in those cells. Furthermore, PC synthesis in quiescent breast cancer cells was slower than in proliferating cells, leading to lower PC levels in the non-proliferating population [4].

The rates of glucose uptake and lactate production have also been monitored with ^{13}C MRS and labelled glucose. Such studies demonstrate that the rate of glycolysis is affected by the proliferation state of the cell, as well as by hormone treatment, with glucose uptake reduced in quiescent cells and in cells treated with tamoxifen [4]. In other cell models, cellular de-differentiation [12] as well as apoptosis [13] were associated with changes in glucose uptake and metabolism that were detectable by MRS.

The chemical shift of the P_i peak enables the determination of pH within a sample. With the use of this information, MRS has been highly instrumental in demonstrating that whereas the extracellular pH of a tumour is acidic, its intracellular pH is in fact neutral to alkaline, leading to a reversed pH gradient in the transformed cell [14,15]. This finding affects the potential usefulness of some chemotherapeutic agents that are designed for optimum activity in an acidic intracellular environment, and some studies aimed at altering the pH gradient have been reported [16]. Another aspect of tumour physiology that can affect response to treatment, particularly radiotherapy, is oxygenation, and ^{19}F MRS of perfluorocarbons can be used in some cases to assess the presence of hypoxic regions within a tumour [15].

Use of MRS in monitoring response to therapy
The response to chemotherapy of experimental tumour models has been reviewed by Steen [17] and Daly and Cohen [18]. They show that untreated tumour growth was associated in several studies with an increase in the PME resonance and a decrease in the signal intensity of energy-rich metabolites. This trend is reversed after chemotherapy. After treatment and tumour shrinkage, a decrease in PME levels has been observed. Furthermore, an increase in NTP levels was reported after response to treatment and was termed by Steen tumour 'activation', a process potentially resulting from improved perfusion to the cells present in the residual shrinking tumour.

The use of MRS to detect the uptake and metabolism of the chemotherapeutic drugs themselves is also possible in some cases, as has been recently reviewed by Griffiths and Glickson [19]. 5-FU, which contains the magnetic res-

onance (MR)-visible ^{19}F nucleus, has been extensively investigated and with ^{19}F MRS it is possible to identify the catabolites of 5-FU in the liver, and to detect the parent drug as well as small peaks originating from the various nucleotides and nucleosides of 5-FU within the tumour itself. ^{19}F MRS has also been used to monitor the conversion of 5-fluorocytosine to 5-FU after transgene expression in a glioma model [20]. Others have been able to detect the expression of a virally transfected gene by monitoring the subsequent MR-visible build-up of a new metabolite in the ^{31}P MRS spectrum [21]. Finally, some studies of specifically labelled drugs such as ^{13}C-temozolamide have also been reported [19], demonstrating that this methodology could also be used to monitor drug pharmacokinetics *in vivo*.

Studies *in vivo*
Use of MRS in differentiating between benign and malignant tumours
Negnedank [22••] comprehensively reviewed the MRS studies *in vivo* performed on different types of human cancer. More recently, concentrating specifically on human breast cancer, Leach *et al* [23••] summarised the finding from nine different ^{31}P MRS studies *in vivo*. In line with the results obtained in model systems and described above, investigations *in vivo* demonstrate large PME and PDE signals in proliferating breast tumours. In 80% of breast cancers, PME signals, composed of both PC and PE, were higher than in normal breast, and PDE signals were higher in 77% of investigated tumours. If the P_i peak is taken as an indicator of pH, these reviews of the literature also demonstrate that breast tumours, like other cancers, show a slightly alkaline pH shift relative to control tissue. More recently, ^{1}H MRS studies performed *in vivo* have also demonstrated an increase in the choline metabolite peak, which reflects choline, PC and GPC levels. Roebuck *et al* [24••] and Kvistad *et al* [25] showed an increase in choline-containing metabolites in 70–80% of breast carcinomas, whereas only 14–18% of benign tumours demonstrated a detectable choline peak. However, choline was also detected in most breast-feeding volunteers.

Use of MRS in monitoring response to therapy
On the basis of the review by Leach *et al* [23••], response to therapy has been associated with a decrease in PME content in 14 of 17 patients, with all non-responding patients demonstrating an increase in PME levels. A further serial study by the same authors of 25 patients undergoing hormone, chemotherapy and radiotherapy treatments showed a significant correlation between a decrease in PME, PDE and total NTP levels and response to therapy as measured by a decrease in tumour volume [23••]. A multi-institutional trial is now in progress to confirm these results by extending localised ^{31}P MRS studies to investigate greater numbers of patients [26].

Considering the use of MR-visible chemotherapeutic drugs to monitor their uptake by the treated tumour, Wolf *et al* [27] recently published results obtained from different tumour types, including 26 cases of breast carcinoma. These studies demonstrated that after a bolus infusion of 5-FU, 'trapping' of the drug within the tumour region for relatively long periods (compared with drug in the blood pool) was strongly associated with tumour response to treatment, with 70% of trappers responding to treatment. None of the non-trappers demonstrated response to treatment.

Future perspectives

The results obtained from tumour extracts, cell models and models of implanted xenografts demonstrate the potential of MRS in assessing surrogate markers of transformation and response to therapy. However, clinical measurements so far have often been limited by the signal : noise ratio as well as the length of time required for measurements. Nevertheless, considerable progress has recently been made in this area. Improved automatic shimming and calibration methods lead to shorter examination times and therefore make spectroscopic studies more acceptable to the patient. The implementation of decoupling in the ^{31}P spectra has already led to an improvement in the separation of PE and PC signals *in vivo* as well as an improvement in signal : noise ratio by providing enhancement by the nuclear Overhauser effect. Recent measurements showing the practicality of ^{1}H spectroscopy in the breast indicate the potential to measure smaller tumours *in vivo* than has been possible with ^{31}P spectroscopy at 1.5 T. This also facilitates integrating such measurements into routine imaging studies because the same coil can be used. Most recently, new developments leading to clinical spectrometers with higher fields should improve both the sensitivity and the resolution of spectra *in vivo*. Together with improved localisation techniques, the ability to acquire signal from smaller voxels should lead to a better separation of tumour and normal tissue, and future studies might be able to generate enough metabolic information to provide both the diagnostic and prognostic parameters required by the clinician.

References

Articles of particular interest have been highlighted as:
* of special interest
** of outstanding interest

1. Degani H, Horowitz A, Itzchak Y: **Breast tumors: Evaluation with**
• **P-31 MR spectroscopy.** *Radiology* 1986, **161**:53–55.
This study assessed the ^{31}P MR spectra of extracts from five benign and nine breast cancer tumours obtained from patients. It demonstrated that both the NTPs and PMEs were consistently higher in the carcinomas compared to controls. Similar results were obtained in [2].

2. Merchant TE, Gierke LW, Meneses P, Glonek T: **^{31}P magnetic resonance spectroscopic profiles of neoplastic human breast tissues.** *Cancer Res* 1988, **48**:5112–5118.

3. Smith TAD, Bush C, Jameson C, Titley JC, Leach MO, Wilman DEV, McCready VR: **Phospholipid metabolites, prognosis and proliferation in human breast carcinoma.** *NMR Biomed* 1993, **6**:318–323.

4. Degani H, Ronen SM, Furman-Haran E: **Breast cancer: spectroscopy and imaging of cells and tumors.** In *NMR in Physiology and Biomedicine*. Edited by Gillies RJ. San Diego: Academic Press; 1994:329–352.

5. Howells SL, Maxwell RJ, Howe FA, Peet AC, Stubbs M, Rodrigues LM, Robinson SP, Baluch S, Griffiths JR: **Pattern recognition of ^{31}P magnetic resonance spectroscopy tumour spectra obtained *in vivo*.** *NMR Biomed* 1993, **6**:237–241.

6. Aboagye EO, Bhujwalla ZM: **Malignant transformation alters membrane choline phospholipid metabolism of human mammary epithelial cells.** *Cancer Res* 1999, **59**:80–84.

7. Bhujwalla ZM, Aboagye EO, Gillies RJ, Chacko VP, Mendola CE, Backer JM: **Nm23-transfected MDA-MB-435 human breast carcinoma cells form tumors with altered phospholipid metabolism and pH: a ^{31}P nuclear magnetic resonance study *in vivo* and *vitro*.** *Magn Reson Med* 1999, **41**:897–903.

8. Gribbestad IS, Petersen SB, Fjosne HE, Kvinnsland S, Krane J: **^{1}H**
• **NMR spectroscopic characterization of perchloric acid extracts from breast carcinomas and non-involved breast tissue.** *NMR Biomed* 1994, **7**:181–194.
This study investigated the high-resolution ^{1}H MRS spectra of uninvolved breast tissue extracts compared with extracts obtained from breast cancer tumours. The proton spectra showed high concentrations of lactate, taurine and succinate, an increase in PC levels and very low phosphocreatine levels in the spectra of tumour extracts.

9. Mackinnon WB, Barry PA, Malycha PL, Gillett DJ, Russell P, Lean CL, Barraclough BH, Bilous M, Mountford CE: **Fine-needle biopsy specimens of benign breast lesions distinguished from invasive cancer *ex vivo* with proton MR spectroscopy.** *Radiology* 1997, **204**:661–666.

10. Podo F: **Tumour phospholipid metabolism**. *NMR Biomed*
• 1999, **12**:413–439.
This is a comprehensive review addressing the following: (1) quantitative MRS measurements of PC, PE, GPC and glycerol phosphoethanolamine in tumours compared with their normal mammalian tissue; (2) possible correlations between the MRS parameters and cell growth and tumorigenicity; (3) current and new hypotheses on the role and interplay of biosynthesis and catabolic pathways of choline and ethanolamine cycles in modulating PE and PC pools.

11. Katz-Brull R, Degani H: **Kinetics of choline transport and phosphorylation in human breast cancer cells; NMR application of the zero trans method.** *Anticancer Res* 1996, **16**:1375–1379.

12. Ronen SM, Volk A, Mispelter J: **Comparative NMR study of a differentiated rat hepatoma and its dedifferentiated subclone cultured as spheroids and as implanted tumors.** *NMR Biomed* 1994, **7**:278–282.

13. Ronen SM, DiStefano F, McCoy CL, Robertson D, Smith TAD, Al-Saffar NM, Titley J, Cunningham DC, Griffiths JR, Leach MO, Clarke PA: **Magnetic resonance detects metabolic changes associated with chemotherapy-induced apoptosis.** *Br J Cancer* 1999, **80**:1035–1041.

14. Griffiths JR: **Are cancer cells acidic?** *Br J Cancer* 1991, **64**: 425–427.

15. Gillies RJ, Bhujwalla ZM, Evelhoch J, Garwood M, Neeman M, Robinson SP, Sotak CH, Van der Sanden B: **Applications of magnetic resonance in model systems: Tumor biology and physiology.** *Neoplasia* 2000, **2**:139–151.

16. Evelhoch JL, Gillies RJ, Karczmar GS, Koutcher JA, Nalcioglu O, Raghunand N, Ronen SM, Ross BD, Swartz HM: **Applications of magnetic resonance in model systems: cancer therapeutics.** *Neoplasia* 2000, **2**:152–165.

17. Steen RG: **Response of solid tumors to chemotherapy monitored by in vivo ^{31}P magnetic resonance spectroscopy: a review.** *Cancer Res* 1989, **49**:4075–4085.

18. Daly PF, Cohen JS: **Magnetic resonance spectroscopy of tumors and potential *in vivo* clinical applications: a review.** *Cancer Res* 1989, **49**:770–779.

19. Griffiths JR, Glickson JD: **Monitoring pharmacokinetics of anti-cancer drugs: non-invasive investigation using magnetic resonance spectroscopy.** *Adv Drug Deliv Rev* 2000, **41**:75–89.

20. Stegman LD, Rehemtulla A, Beattie B, Kievit E, Lawrence TS, Blasberg RG, Tjuvajev JG, Ross BD: **Noninvasive quantitation of cytosine deaminase transgene expression in human tumor xenografts with *in vivo* magnetic resonance spectroscopy.** *Proc Natl Acad Sci USA* 1999, **96**:9821–9826.

21. Walter G, Barton ER, Sweeney HL: **Noninvasive measurement of gene expression in skeletal muscle.** *Proc Natl Acad Sci USA* 2000, **97**:5151–5155.

22. Negendank W: **Studies of human tumors by MRS: a review.**
•• *NMR Biomed* 1992, **5**:303–324.
This is the most comprehensive review so far of all tumour MRS studies *in vivo*. The literature describing ^{31}P, ^{1}H, ^{13}C, ^{23}Na and ^{19}F studies is reviewed. It concludes that cancers have high phospholipid metabolites and a cellular pH more alkaline than normal. It also shows that some correlation with response to treatment is emerging.

23. Leach MO, Verrill M, Glaholm J, Smith TAD, Collins DJ, Sharp JC,
• Ronen SM, McCready VR, Powles TJ, Smith IE: **Measurements of human breast cancer using magnetic resonance spectroscopy: a review of clinical measurements and a report of localized ^{31}P measurements of response to treatment.** *NMR Biomed* 1998, **11**:314–340.
This review concentrates primarily on results obtained from ^{31}P MRS studies of breast cancer. In addition to reviewing the literature, the authors also show results from 25 serial studies of patients undergoing different forms of therapy for breast cancer. Their results demonstrate a correlation between tumour response as measured by shrinkage and changes in PME, NTP and total phosphate levels. A correlation with metabolite levels at week three after treatment is also shown.

24. Roebuck JR, Cecil KM, Schnall MD, Lenkinski RE: **Human breast**
•• **lesions: characterization with proton MR spectroscopy.** *Radiology* 1998, **209**:269–275.
This study was concerned with assessing breast tumours by ^{1}H MRS. It demonstrated that spatially localised ^{1}H MRS can provide sufficient sensitivity and spectral resolution at 1.5 T to detect choline in seven of ten human malignant breast lesions. No choline was detected in six of seven patients with benign tumours.

25. Kvistad KA, Bakken IJ, Gribbestad IS, Ehrnholm B, Fjosne HE, Haraldseth O: **Characterization of neoplastic and normal human breast tissues with *in vivo* ^{1}H MR spectroscopy.** *J Magn Reson Imaging* 1999, **10**:159–164.

26. Arias-Mendoza F, Brown TR, Charles HC, Zakian K, Schwarz A, Doyle V, Nelson SJ, Rijkema M, Glickson JD, Evlehoch JL: **Methodological standardization for a multi-institutional in vivo trial of localized ^{31}P MR spectroscopy in human cancer research [abstract].** *Proc Int Soc Magn Reson Med* 1999, **3**:1585.

27. Wolf W, Waluch V, Presant CA: **Non-invasive ^{19}F-NMRS of 5-fluorouracil in pharmacokinetics and pharmacodynamic studies.** *NMR Biomed* 1998, **11**:380–387.

28. Smith TAD, Glaholm J, Leach MO, Machin L, Collins DJ, Payne GS, McCready VR: **A comparison of in vivo and in vitro ^{31}P NMR spectra from human breast tumours: variations in phospholipid metabolism.** *Br J Cancer* 1991, **63**:514–516.