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Magnetic Resonance Spectroscopy and Imaging Guidance in Molecular Medicine: Targeting and Monitoring of Choline and Glucose Metabolism in Cancer

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Abstract

Magnetic resonance spectroscopy (MRS) and spectroscopic imaging (MRSI) are valuable tools to detect metabolic changes in tumors. The currently emerging era of molecular medicine, which is shaped by molecularly targeted anticancer therapies combined with molecular imaging of the effects of such therapies, requires powerful imaging technologies that are able to detect molecular information. MRS and MRSI (MRS/I) are such technologies that are able to detect metabolites arising from glucose and choline metabolism in noninvasive *in vivo* settings and at higher resolution in tissue samples. The roles that MRS/I plays in diagnosing different types of cancer as well as in early monitoring of tumor response to traditional chemotherapies are reviewed. Emerging roles of MRS/I in the development and detection of novel targeted anticancer therapies that target oncogenic signaling pathways or targets in choline or glucose metabolism are discussed.

Keywords

Magnetic resonance spectroscopy; MRS; metabolism; glucose; choline

1. Introduction

Although a lot of progress has been made in diagnosing cancers early and improving available treatment options, cancer still is a devastating disease that claims one in four death in the United States (1). A unique problem with anticancer therapies is that cancer cells can adapt to these therapies and survive them, leading to treatment resistance. This problem arises due to the genomic instability of cancer cells, which is additionally triggered by their cooperative interaction with stromal cells and the tumor microenvironment (TME). Together, cancer cells, stromal cells, and the TME progress to form a primary tumor, invade surrounding normal tissue, and give rise to metastatic nodules at distant sites. To overcome the problem of resistance to treatment, and to render anticancer chemotherapies less toxic, tremendous research efforts have been invested in the development of molecularly targeted

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anticancer therapies. The investigation of strategic molecular targets has uncovered successful targets in oncogenic signaling pathways, such as receptor tyrosine kinases and their downstream effectors, as well as targets in key pathways affecting proliferation, apoptosis, angiogenesis, invasion and metastasis, previously defined as the hallmarks of cancer (2). Recent preclinical and clinical cancer research findings have clearly shown that personalized molecular targeting is necessary for the successful treatment of cancer, in which more than one critical target is being hit at once.

One important biological outcome that results from the interplay between genetically altered tumor cells and the physiological TME is the altered tumor and cancer cell metabolism. Glucose metabolism and choline metabolism are significantly different in tumors. This is a result of changes in oncogenic signaling pathways that lead to changes in expression and/or activity of enzymes involved in glucose and choline metabolism. The altered tumor metabolism can be detected with noninvasive imaging modalities such magnetic resonance (MR), positron emission tomography (PET), and single photon emission tomography (SPECT), which are well suited to detect metabolic biomarkers or surrogate markers in cancer diagnosis and anticancer treatment. In this review we have focused on noninvasive magnetic resonance spectroscopy (MRS) and spectroscopic imaging (MRSI) and their roles in guiding molecular medicine by playing important roles in molecular target discovery, molecular targeting, diagnosis, and noninvasive monitoring of choline and glucose metabolism in cancer to assess treatment success or failure.

MRS is very helpful in studying metabolism because the signals of different metabolites occur at different chemical shifts. The aberrations in the genome and proteome of cancer cells result in their unique metabolic phenotype. The cancer metabolic phenotype is characterized by high glucose uptake, increased glycolytic activity and lactate production, decreased mitochondrial activity, low bioenergetic status, and aberrant phospholipid metabolism (3-5). Tissue-specific metabolites such as N-acetyl aspartate in the brain, and citrate in the prostate, show an MRS-detectable decrease as a result of an expanding cancer cell population combined with a reduction of the normal tissue fraction (5-7). Proton or ^{31}P MRS or MRSI detection of these endogenous metabolites can help diagnose cancer (8-15), and monitor anticancer therapy in some cases, as outlined in this review. The fact that MRS/I is noninvasive makes it such a powerful technique because this opens up the possibility to perform dynamic monitoring of tumor metabolism in patients. However, the spectral resolutions achieved *in vivo* are relatively low at currently approved clinical magnetic field strengths, making it necessary in some cases to observe combined overlapping signals *in vivo*, such as the total choline containing signal at 3.2 ppm. *Ex vivo* applications of MRS provide a much higher spectral resolution, and novel techniques based on magic angle spinning that can avoid extraction of the tissue have evolved over the past decade. Such high-resolution (HR) magic angle spinning (MAS) MRS techniques are currently being implemented in the diagnostic clinical workflow following biopsy or surgical resection of tumors, and will be further explained in this review.

MRS studies on the aberrant cancer cell metabolism have resulted in the identification relevant enzymes as novel anticancer targets (4, 16-19). The total choline (tCho) signal is already being explored in the clinical setting as MR biomarker for assisting in tumor diagnosis and early monitoring of tumor response to anticancer therapy (20-22). Molecular targeting of targets in choline phospholipid metabolism can be noninvasively detected and imaged by MRS and MRSI (MRS/I). Pharmacological and molecular approaches to target choline kinase, a key enzyme in choline metabolism, are currently being developed (16, 23), and are discussed in detail below. In this review, we have described some recent biomedical MRS applications of ^1H , ^{31}P , and ^{13}C MRS in preclinical models of cancer, and have

outlined new developments, such as ^{13}C hyperpolarization to improve the MR detection sensitivity of ^{13}C -labeled substrates.

2. Key molecules driving the altered choline metabolism in cancer

MRS is especially useful in studying metabolism in heterogeneous tissues such as tumors with its ability to identify different compounds by their chemical shifts. MR detects radiofrequency (RF) signals emitted after MR active nuclei such as ^1H , ^{31}P , ^{13}C , and ^{19}F are excited by external alternating magnetic fields. MR spectra can be acquired with or without spatial localization. In the absence of spatial information, the signal is acquired from the entire sensitive region of the coil that detects the RF signal. Localized MRS can either be acquired from a single volume element (single-voxel), or from multiple voxels (multi-voxel) to generate images of the spatial distribution of metabolites, referred to as MRSI. When measuring such localized MR spectra, phase-encoding gradients are added to the MRS or MRSI (MRS/I) sequence to generate images of signals with spatially encoded chemical shift information. This phase encoding can be applied in one, two, or three spatial dimensions, and its application is useful in deciphering metabolic distributions in heterogeneous tissues such as tumors.

Elevated metabolites in choline phospholipid metabolism in cancer, which can be noninvasively detected and imaged by MRS/I (16, 23), present unique targets to exploit for molecular targeting. Malignant transformation has been observed to alter the profile of the choline compounds glycerophosphocholine (GPC) and phosphocholine (PC) in breast (24) and ovarian (25) cancer cells, which was detected by using MRS/I. GPC is the predominant signal in nonmalignant breast and ovarian epithelial cells, whereas PC displays the highest concentration in ovarian and breast cancer cells (24, 25). Prostate cancer cells contain elevated PC and GPC levels (26). The choline phospholipid metabolites typically detected in high-resolution ^1H MR spectra are free choline (Cho), PC, and GPC. Similarly, in high-resolution ^{31}P MR spectra, signals from PC and phosphoethanolamine (PE), and GPC and glycerophosphoethanolamine (GPE) are detected. In clinical settings, ^1H or ^{31}P MRS or MRSI detection of these choline metabolites has been proven useful in the diagnosis of cancer (8-15), and in monitoring the response of tumors to anticancer therapy (20-22), as detailed in subsequent sections.

2.1 Magnetic resonance spectroscopy techniques for detecting and differentiating cancer, and monitoring anticancer therapy, based on choline metabolites

Proton MRS signals from water-soluble choline metabolites arise between 3.2 – 3.3 ppm from the nine chemically equivalent protons in the choline- $\text{N}(\text{CH}_3)_3$ groups. In Figure 1, color-coded ^1H nuclei are displayed in the chemical structure formula that give rise to the MR signals that are shown with the same color-code in representative MR spectra. Because nine protons contribute to this signal, it displays a higher signal intensity than the ^1H signals in the methylene groups of the same choline metabolites, which only contain two equivalent protons each (see Figure 1). Using 3-(trimethylsilyl)propionic-2,2,3,3- d_4 acid (TSP) as a chemical shift reference at pH 7.4, free choline is detected as a peak at 3.21 ppm, PC at 3.23 ppm, and GPC at 3.24 ppm, in high-resolution (HR) ^1H MR spectra of cell or tissue extracts, or in HR magic angle spinning (MAS) MR spectra. The chemical structures of Cho, PC, and GPC are shown in Figure 1. It is not possible to resolve Cho, PC, and GPC *in vivo*, even at higher magnetic field strengths, because of the broader line widths that result from restricted molecular motion and magnetic field inhomogeneities. As an alternative, a single unresolved peak arising from total choline containing compounds (tCho), comprising of these three signals is detected, as shown in Figure 1. Multi-voxel *in vivo* ^1H MRSI of one or multiple slice(s) through a region of interest provides multiple spectra from a slice or volume of tissue that can be processed to obtain the spatial distribution of tCho or other metabolites.

2.1.1 ^1H MRS/MRSI in cancer detection and treatment based on choline metabolites—Clinical multi-center trials are currently underway to establish single-voxel ^1H MRS or multi-voxel ^1H MRSI as a tool for cancer detection. Numerous of studies investigated the feasibility and diagnostic value of combined MRI and MRS/I in the detection of cancer (9, 13, 14, 27-31). Several research groups investigated the feasibility of combined dynamic contrast enhanced (DCE) MRI and MRS in evaluating breast lesions (14, 30). In a study by Jacobs *et al*, five patients with breast carcinoma and four with benign lesions were evaluated (30). Persistent enhancement of lesion time intensity, which describes how the inspected tissues absorb and release the contrast agent over the period of time that coincides with the MRI examination, was found in two benign cases, plateau enhancement in two of four benign and four of five malignant lesions, and one malignant case exhibited a washout pattern (30). The tCho signal-to-noise ratio (SNR) was significantly different ($P < 0.003$) between benign and malignant lesions (30). A combination of DCE MRI and MRS proved feasible, and improved specificity compared to either modality alone (30). Throughout the past few years, several studies with hundreds of breast cancer patients have demonstrated that an increased MRS-detected tCho signal that overlaps with the contrast-enhancing breast lesion in DCE MRI can be used to identify malignant breast tumors and distinguish them from benign breast lesions (14, 31, 32). Recent studies of prostate cancer (28, 29) compared the area under the receiver operating curve (ROC) of combined MRI and MRS, MRI alone, and MRS alone. These studies found that the combination of MRI and MRS yielded superior diagnostic results than either modality alone, in terms of sensitivity (72.3%), specificity (92.6%) and accuracy (80.1%) of combined MRI and MRS for detection of prostate cancer. Combining whole prostate qualitative MRI with MR spectral estimation was shown in previous research studies to be feasible in routine clinical practice (28, 29).

Many studies use peak area ratios as a criterion to describe the changes in metabolic profiles in cancer detection. The advantage of using ratios is that the effects of cell density variations that can affect the calculation of metabolite concentrations are canceled against each other when calculating a ratio. MRS of the prostate provides information of the cellular metabolites of the gland. Normal prostate tissue contains high levels of citrate, occurring at chemical shifts of 2.50 ppm and 2.72 ppm {van der Graaf, 1996 #412}. In prostate cancer (33), the citrate level is diminished or undetectable and the tCho level is elevated (Figure 2). The total creatine (tCr) peak, usually around 3.0 ppm, is very close to the tCho peak (3.2 ppm) in these *in vivo* MR spectra and may not always be separable. The tCr signal is assumed to remain unaltered between healthy and cancerous tissue. For this reason and practical purposes, the ratio of tCho plus tCr over citrate [choline + creatine]/citrate (CC/Ci) is used for spectral analysis to make it feasible for clinical practice (33). ^1H MRS of brain tumors compared to normal brain detected decreased N-acetylaspartate (NAA) and frequently increased tCho. An increase in the ratio of tCho to NAA (tCho/NAA) was highly correlated with increased tumor malignancy and can be used as a noninvasive index of tumor grade (9, 34-36).

Results of numerous *ex vivo* (37-39) and *in vivo* (40, 41) studies have shown that neoplastic breast tissue contains elevated levels of tCho. Since the sensitivity of *in vivo* MRS is limited, adding a quantitative analysis of ^1H MRS, such as calculating the tCho concentration of the lesions (14), to the breast imaging examination could help to improve the radiologist's ability to characterize malignant breast lesions. Two referencing strategies have been used for converting measured signal amplitudes into concentrations. The first method is to use external referencing, in which the *in vivo* measurement is compared with a measurement from an external phantom of known concentration (42, 43). The second referencing strategy proposed for breast MRS is to use the water peak from a non-suppressed spectrum from the

same voxel as an internal reference. The tCho concentration is calculated by comparing the tCho and water signal intensities and correcting for the differences in relaxation (40, 44).

MRS/I cannot only help with the diagnosis of cancers in the clinic, but also monitor clinically approved anticancer treatments. Multiple studies have identified changes in tCho as having a high likelihood of detecting early response to treatment in breast cancer (21, 45, 46). Neoadjuvant chemotherapy (NACT) is the standard treatment for patients with inoperable locally advanced breast cancer (LABC). A recent study (22) compared changes in tCho concentration and in tumor size at follow-up after NACT between patients, who achieved pathologic complete response (pCR) and those who did not, by using MRI and quantitative ^1H MRS (22). Figure 3 shows the maximum intensity projection MR images (left) and corresponding MR spectra (right) from a 40-year-old patient who achieved pCR (22). In this study, patients who had a greater reduction in tCho compared with changes in tumor size were more likely to achieve pCR (22). Another recent study (47) was designed to investigate the changes in the signal-to-noise ratio (SNR) of the tCho resonance (ChoSNR) using MRSI and an anatomical parameter, such as the volume of the tumor, in patients with LABC, and then determined the potential clinical utility of these parameters in the assessment of tumor response during the various stages of NACT. This study concluded that ChoSNR might serve as a useful parameter in predicting tumor response in the absence of the absolute tCho concentration (47). ChoSNR gave a sensitivity of 85.7% with a specificity of 91% (47). An early response of prostate cancer to hormone-deprivation therapy (48, 49) and cryosurgery (50) was detected by a decrease in [tCho/citrate] ratios quantified from ^1H MR spectra. Anticancer treatments leading to apoptosis or necrosis induced characteristic changes in choline phospholipid metabolites (51). An early response to therapy in lymphomas was also associated with a reduction of tCho (52). Some cancers, e.g. cervical cancer, did not exhibit a change in the tCho signal following neoadjuvant therapy, followed by surgical resection of the tumors (53). However, these cervical cancers displayed a reduced tumor volume along with decreased triglyceride signal and no survival advantage associated with the reduced tumor volume or decreased triglycerides (53).

2.1.2 HR MAS ^1H MRS in cancer detection and treatment based on choline metabolites—The relatively new applications of *ex vivo* high-resolution MR technique HR MAS ^1H MRS to fresh tissues, which can be implemented in the clinical workflow, is currently gaining importance in diagnosis and treatment monitoring of cancer. HR MAS ^1H MRS is well suited to examine intact biological tissue *ex vivo* at high spectral resolution (7, 54-56). In the clinic, HR MAS ^1H MRS can be useful for analyzing the metabolome of biopsy specimens prior to pathological classification (7, 54-56). In HR MAS ^1H MRS, the solid sample is spun at a frequency of up to 12 kHz at the magic angle of θ_m , which is 54.74° with respect to the direction of the magnetic field. At this magic angle, where $(3\cos^2\theta_m-1)/2$ approaches zero, residual dipolar interactions average out, and therefore the normally broad lines become narrower and the spectral resolution increases for better identification and analysis of the spectrum (7, 54-56). One major advantage of this technique is that, unlike in HR MRS of tissue extracts, the HR MAS ^1H MRS-analyzed tissue can be used for subsequent histologic, biochemical, and genetic analyses (7, 54-57). The high spectral resolution of HR MAS ^1H MR spectra makes their data analysis more challenging. A combination of different multivariate data analysis methods such as principal component analyses or neural network applications is typically used to process these rich data sets (57). A recent study demonstrated that HR MAS ^1H MRS was able to detect the metabolic phenotype of tumors, demonstrating a marked metabolic difference between healthy breast and breast tumor tissue evident by clearly separated score plots of three principal components (57). Absolute metabolite quantification can also be performed from HR MAS ^1H MR spectra either alone or in combination with multivariate data analysis (58). Knowledge of absolute tumor metabolite levels can help understand the disease process on a

biochemical and molecular level. A known amount of TSP can be added to the samples for quantification, or a pre-calibrated Electronic REference To access In vivo Concentrations (ERETIC) can be applied to HR MAS ^1H MRS to quantify metabolites in intact cancer samples (58). HR MAS ^1H MRS has a great potential to be used clinically as a complement for histopathological analysis, for detecting and characterizing cancers (57, 58).

2.1.3 ^{31}P MRS/MRSI in cancer detection and treatment based on choline metabolites—Phosphorus-containing choline metabolites can be detected with ^{31}P MRS, as demonstrated in Figure 1. Following chemical shift calibration to a reference compound such as methylene diphosphonic acid at 18 ppm, a signal from PC at 3.9 ppm and GPC at 0.5 ppm are observed in HR ^{31}P MR spectra of cell or tumor extract, as shown by color-coded signals and their corresponding ^{31}P nuclei with the same color-code in the chemical structures. In the *in vivo* setting, a mixed phosphomonoester (PME) signal containing unresolved PC and phosphoethanolamine (PE) resonances, and a mixed phosphodiester (PDE) signal containing unresolved GPC and GPE resonances are observed in ^{31}P MR spectra (see color-coding in Figure 1).

Since ^1H MRS has a higher sensitivity and a higher availability of a scanning standard compared with ^{31}P MRS, it is much more popular on clinical scanners. However, a multi-institutional group has been created to investigate the utility of *in vivo* ^{31}P MRS in studying human cancers *in vivo* (59). The standardization of the acquisition protocol for *in vivo*-localized ^{31}P MRS across different institutions has resulted in comparable *in vivo* data, decreasing the possible problems related to a research study carried out under a multi-institutional setting. A recent clinical study demonstrated the feasibility of partially resolving PC from PE, and GPE from GPC by using ^1H -decoupled ^{31}P MRS at 1.5 T (60) and at the higher field strength of 3T that is also clinically available. A large-scale multi-center trial across nine institution using ^{31}P MRS for non-invasive monitoring of chemotherapy of solid non-Hodgkin's lymphoma (NHL) pre- and post-treatment, was performed on either General Electric (GE) or Siemens clinical 1.5T MR scanners (61). In this multi-center ^{31}P MRS trial, the PME/NTP ratio significantly decreased following treatment in complete ($P < 0.001$) and partial ($P < 0.05$) responders, but not in non-responders ($P > 0.1$) (61). The PME/NTP ratio in the pre-treatment ^{31}P MR spectra also correlated with the treatment outcome, indicating that the PME/NTP ratio can serve as a significant predictor of long-term clinical response and time-to-treatment failure in NHL.

Two recent studies reported a novel ^1H to ^{31}P polarization transfer method by applying chemical shift selective refocusing pulses at 3T, which can achieve a more than twofold increase of SNR compared to direct ^{31}P MRS methods (62, 63). This selective refocused insensitive nuclei-enhanced polarization transfer (sRINEPT) method provided baseline-resolved PE, PC, GPE, and GPC peaks in human brains (62, 63). The sRINEPT method allowed to generate a three-dimensional ^{31}P MRSI data set with good SNR from voxel sizes of 17 cm^3 within a clinically acceptable measurement time of 17 minutes (63). Evaluation of intact tissue samples from experimental tumors has been performed using ^1H -decoupled ^{31}P HR MAS MRS (64, 65). The concentrations of the well-resolved tissue metabolites showed good agreement with concentrations measured from extracts of the same pieces of tissue (64, 65).

2.1.4 ^{13}C MRS/ MRSI in cancer detection and treatment based on choline metabolites—For studies of choline metabolites, ^{13}C MRS can be useful to detect ^{13}C -labeled metabolites following administration of ^{13}C -labeled free choline or methionine as substrates in cancer cells and solid tumors (23, 66-70). The ^{13}C label in either the $-\text{CH}_2$ or the NCH_3 groups of free choline can be followed with ^{13}C MRS to detect the incorporation of a ^{13}C label in the downstream metabolites and products, such as PC and GPC.

Quantitative flux rates can be calculated by metabolic modeling. Carbon-13 MRS methods that attempt to detect the ^{13}C directly suffer from relatively low sensitivity. However, magnetization transfer techniques can improve the ^{13}C MRS sensitivity. Examples of such magnetization transfer techniques that can drastically improve the detection sensitivity of ^{13}C nuclei are nuclear Overhauser effect (NOE) methods, heteronuclear cross-polarization (HCP) experiments (71), and indirect inverse detection methods (72). In NOE techniques, the ^{13}C signals are enhanced because magnetization is transferred from neighboring protons through space using dipole-dipole spin coupling, or through chemical bonds using the J-coupling between ^{13}C and ^1H spins. Direct ^{13}C detection methods using magnetization transfer to enhance ^{13}C signals are NOE, distortionless enhancement by polarization transfer (DEPT), insensitive nuclei enhanced by polarization transfer (INEPT), and HCP. Indirect detection schemes, in which the magnetization is transferred from ^1H to ^{13}C and then back to ^1H , are heteronuclear multiple quantum coherence (HMQC) and heteronuclear single quantum coherence (HSQC) methods. Carbon-13 signal detection in HMQC and HSQC is done indirectly as ^1H frequencies, which significantly enhances the sensitivity due to the higher gyromagnetic ratio γ of ^1H (72).

2.2 Altered enzymes in choline metabolism of cancer

^1H MRS consistently detects significant differences in choline phospholipid metabolites of malignant *versus* benign breast lesions. It is critically important to understand the molecular causes underlying these metabolic differences, because this may identify novel targets for anticancer therapy selective for cancer cells. The major molecular causes for the increased PC and tCho levels in cancer cells and tumors include an increased expression and activity of choline kinase alpha (CHK- α) (16, 18, 23, 73-76), a higher rate of choline transport (77, 78), and increased activities of phosphatidylcholine (PtdCho)-specific phospholipase C (PtdCho-PLC) (25, 79) and D (PtdCho-PLD) (25, 80-82), and several phospholipases A₂ (83, 84). Other enzymes in the choline phospholipid metabolic pathways are in the process of investigation (85). All these enzymes participate in the biosynthetic and breakdown pathways of the major membrane phospholipid phosphatidylcholine (PtdCho) (23) as shown in the schematic in Figure 4.

CHK- α is responsible for the generation of PC from its precursor Cho in the biosynthesis pathway of PtdCho. Both CHK- α (16, 18) and its product PC (24) have been recently reported as essential molecules in cell proliferation and transformation. One study correlated CHK- α expression and activity with clinical features in breast cancer patients, demonstrating that CHK- α is overexpressed in 17% of human mammary carcinomas, and its activity is increased in 38.5% of the analyzed breast tumors (18). The levels of CHK- α expression were also explored in both normal and tumoral cell lines derived from lung, colon, and prostate samples (86). In keeping with the role of CHK- α and PC in malignant transformation, CHK- α expression was found up-regulated at a high percentage in human tumor-derived cell lines as well as in different human tumoral tissues (86).

CTP:phosphocholine cytidyltransferase (CCT) is the rate-limiting enzyme in the biosynthetic pathway of PtdCho and its downregulation may be contributing to the elevated PC and tCho levels in cancer cells as well (87, 88). Within the breakdown pathways of PtdCho catabolism, PtdCho-PLC activation has been implicated in contributing to the aberrant choline phospholipid metabolism in cancer (25, 79, 89-91). The overall activities of PtdCho-PLC and PtdCho-PLD were higher in human ovarian carcinoma cell lines as compared with normal or immortalized ovary epithelial cells (25, 79). CHK- α (4, 16-19, 92-94) and PtdCho-PLD (81) and PtdCho-PLC (25) have been explored as molecular targets for anticancer therapy as outlined in section 2.4. Additional insight into the enzymes that are altered in choline metabolism in cancer can be found in recent reviews (4, 95, 96).

2.3 Oncogenes and signaling pathways arriving at cancer-phenotypic endpoints of choline metabolism

Multiple growth factors, cytokines, oncogenes, and chemical carcinogens have the ability to regulate and impact upon the enzymes in choline phospholipid metabolism (4, 97, 98). Cancer cells display distinct phenotypic endpoints in choline metabolism characterized by high PC, relatively high GPC, and high tCho as a result of deregulated oncogenic signaling pathways that impact upon the enzymes involved in choline metabolism, some of which are outlined below. While GPC is the dominating signal in normal breast and ovarian epithelium, PC is higher than GPC in ovarian and breast cancer cells (24, 25). Carcinogenesis of prostate epithelium results in elevated PC and GPC levels (26).

Some of the most important players in deregulated oncogenic signaling cascades are mutant *ras* oncogenes. Transformation with the *H-ras* oncogene resulted in an inhibitory effect on CCT (87, 88) as shown in Figure 4. *Ras* oncogene transformation of NIH 3T3 fibroblasts decreased PtdCho-PLC activity, and correlated with PtdCho-PLC translocation from the cytosol to the plasma membrane (90). Gain-of-function mutations in *Ras* can activate PtdCho-PLD, mediated by positive regulatory pathways through RAL-GDS and phosphatidylinositol 3-kinase (PI3K), and negative feedback signaling through RAF-1 and RAL-GDS (99). Mutant *ras* oncogene transformation caused an increase in CHK- α activity, which correlated with increased PC levels (100). *Ras*-mediated regulation of CHK- α also involved RAL-GDS and PI3K (101). Taken together, the drastic increase that can be observed in cellular PC levels following mutant *ras*-oncogene transformation (88, 102) is most likely due to the combined effects of oncogenic RAS protein regulation of CHK- α (101, 103), CCT (87, 88), PtdCho-PLD (99), and PtdCho-PLC (90) activity.

Mitogen activated protein kinases (MAPK) such as extracellular signal-regulated kinases (ERK), which are activated by phosphorylation, are frequently deregulated in several types of cancer, and are currently being explored as drug targets (104). Inhibition of ERK1/2 phosphorylation resulted in a significant drop in the PC levels of cancer cells (104), indicative of a positive correlation between ERK activation and high PC levels as shown in Figure 4. CHK- α silencing significantly reduced phosphorylated ERK levels, and was able to attenuate MAPK signaling (105). PC may serve as a noninvasive pharmacodynamic MRS-detected marker for monitoring MAPK signaling blockade (104).

The PI3K pathway is deregulated in most cancer, and is a major target for cancer drug development (106, 107). PI3K inhibition resulted in a concentration- and time-dependent decrease in PC and tCho, most likely due to a decreased in CHK- α protein expression levels (106, 107), as indicated in Figure 4. MRS monitoring of the effects of PI3K inhibitors may provide a noninvasive pharmacodynamic biomarker for PI3K inhibition (106, 107). The reversal of this pathway was demonstrated when CHK- α silencing decreased cellular PC, PtdCho, and phosphatidic acid levels as well as signaling through the PI3K/AKT pathway, with reduced levels of phosphorylated AKT detected following CHK- α silencing (105).

A recent study has demonstrated that the transcription factor hypoxia-inducible factor (HIF)-1 was able to bind to the putative *Chk- α* promoter regions and increase CHK- α expression along with cellular PC and tumor tCho levels detected by MRS/I (108). HIF-1 alpha is stabilized under hypoxic conditions, which can frequently arise within the tumor microenvironment, and enables cancer cells to adapt to hypoxic conditions by inducing the expression of a multitude of genes. Additional HIF-1 responses in cancer cells are discussed in section 3.

Expression of the *Her-2* oncogene in nonmalignant human mammary epithelial cells significantly increased PC and tCho levels (24). An interesting link was recently revealed

between PtdCho-PLC and *Her-2*, where inhibition of PtdCho-PLC was able downregulate HER-2 overexpression on the plasma membrane of breast cancer cells (109). Additional oncogenic signaling pathways affecting enzymes in choline metabolism are currently under intense investigation and were recently reviewed in (4, 95, 96).

The connection between oncogenic signaling pathways and choline metabolism makes it possible to use the tCho and PC signals as surrogate markers for molecular therapies that target these oncogenic signaling pathways. For example, a decrease of tCho and PC was observed upon targeting mitogen activated protein kinase (MAPK) (104), fatty acid synthase (110), and BCR-ABL tyrosine kinase (111). However, PC levels increased following histone deacetylase (HDAC) inhibition (112), demonstrating that the treatment effects on choline metabolism when targeting signaling pathways must be carefully examined before designating noninvasive surrogate markers such as PC and tCho for these novel molecular therapies. An overview of the key oncogenic pathways affecting enzymes in choline metabolism is shown in the right part of Figure 4. Several oncogenic signaling pathways that affect choline phospholipid metabolic enzymes can also modulate the expression and/or activity of enzymes in glucose metabolism as outline in section 3.4.

2.4 Targeting of choline metabolism for cancer therapy

CHK- α (4, 16-19, 92-94) and PtdCho-PLD (81) and PtdCho-PLC (25) have been explored as molecular targets for anticancer therapy, and have been silenced or inhibited in studies of MRS-monitored, targeted anticancer therapies. Downregulation or inhibition of CHK(- α) or PtdCho-PLD can be detected noninvasively with ^{31}P or ^1H MRS (25, 79, 92, 93). Preclinical anticancer treatments with CHK inhibitors caused tumor growth arrest and apoptosis (19, 92, 94), and silencing CHK- α with siRNA significantly reduced breast cancer cell proliferation and increased differentiation (16). A recent study showed that longitudinal noninvasive single-voxel ^{31}P MRS monitoring of tumoral PC and PME levels was feasible to assess successful gene therapy with an intravenously injected lentiviral vector, which was able to deliver CHK- α -specific short-hairpin RNA (shRNA) in a preclinical breast cancer model (93). A more extensive review of targeting choline metabolism for cancer therapy can be found in recent reviews on his topic (4, 95, 96).

3. Key molecules in the altered glucose metabolism in cancer

A common feature across most, if not all, cancer types is abnormal glucose metabolism. Cancer cells typically exhibit a high rate of glucose uptake and utilization. In contrast to normal cells, glucose is converted to lactate also under aerobic conditions. This phenomenon was first studied by Otto Warburg in the 1920's and is therefore known as the Warburg effect (113, 114). As the connections between malignant transformation and cellular metabolism on the molecular level gradually have been uncovered, this field of cancer research has enjoyed increased attention in recent years. This research has demonstrated upstream regulation of several metabolic pathways by major oncogenes such as *Myc* and *PI3K/Akt*, and that abnormalities in these metabolic systems therefore are interconnected (115, 116). These metabolic features of cancer cells both facilitate cell survival in hypoxic environments and provide a basis for increased cell proliferation both with respect to energy demand and cellular building blocks. Another factor that enables the cancer cells to adapt to hypoxic conditions is the hypoxia-inducible transcription factor (HIF-1). Stabilization of this factor induces the expression of several genes involved in glucose metabolism, angiogenesis and other mechanisms.

The high uptake of glucose and the extensive production of lactate make it feasible to use MRS in the assessment of glucose metabolism. Both glucose and lactate can be observed in proton spectra. In ^{13}C MRS, assessment of glucose and lactate is typically facilitated

through the use of isotopically enriched substrate. The most widely used substrate is [1-¹³C] glucose, which give rise to two separate resonances from the anomers α -glucose (94.8 ppm) and β -glucose (98.7 ppm). The isotopical enrichment is retained throughout glycolysis, resulting in the formation of [3-¹³C] lactate (22.8 ppm) and [3-¹³C] alanine (19.0 ppm). In addition, [2-¹³C] acetyl-CoA enters the TCA cycle giving rise to [4-¹³C] glutamate and other enriched TCA intermediates.

MRS provides unique possibilities for noninvasive studies of the flux through metabolic pathways in tumor cells. The microenvironment of solid tumors and the regulation of metabolic pathways are closely associated. MRS has an advantage compared to classical biochemical methods in that both spatial and temporal variation in metabolic activity can be studied *in vivo*. This has led to clinical exploitation of the altered glucose metabolism in cancer management (117, 118), but MRS is also a versatile research tool. Glucose metabolism in cancer cells has been studied for more than two decades using ¹³C-labeled substrates (119, 120), allowing detailed description of the metabolic fate of glucose in various experimental systems.

3.1 Magnetic resonance spectroscopy techniques for detecting and differentiating cancer based on glucose metabolism

Glucose metabolism in cancer can be studied using various MRS techniques. High resolution MRS can be applied to cell cultures, tissue samples and extracts. Proton spectroscopy is routinely used in cancer diagnosis and management, and assessment of lactate production is an important parameter in brain cancer. Other metabolites in the glycolysis are present in low concentrations and are seldom considered as biomarkers in proton spectroscopy. Most studies of the glycolytic pathway use ¹³C MRS for tracing the metabolic fate of [1-¹³C] glucose or other isotopically enriched substrates. ¹³C MRS has also been used to study glucose metabolism *in vivo* (121, 122). Following administration of ¹³C-enriched substrates to tumor-bearing animals, the fate of the substrates can be studied taking tumor microenvironmental factor into account. However, ¹³C MRS is not yet routinely employed in clinical diagnosis or management of cancer. This may change as new spectroscopic techniques, such as hyperpolarised ¹³C MRSI, are further developed.

3.1.1 Clinical ¹H MRS/I of glucose metabolism—Clinical ¹H MRS has proven valuable in assessment of several cancer types due to tissue-specific changes in metabolite pattern (123). However, assessment of glycolysis using ¹H MRS is difficult in several cancer types, as concentrations of most glycolytic metabolites cannot be measured. The exception is lactate, which may be present in measurable concentrations in cancer. Therefore, ¹H MRS is widely used in diagnosis, staging and treatment monitoring of brain cancer. Lactate is present in minute concentrations in normal brain tissue, but reaches high concentrations in cancer due to abnormal glycolytic activity. Together with decrease in N-acetyl-aspartate (NAA) due to displacement of healthy neurons, lactate is a functional biomarker for brain cancer.

Typically, ¹H MRS/I is used in combination with MR imaging for diagnostic purposes. The relative intensities of lactate, choline and NAA can be used to distinguish between cancer, benign lesions and post-surgery lesions in the brain. High levels of lactate are associated with high-grade tumors, allowing grading of brain tumors based on metabolite concentrations (124). High resolution MRSI may also aid in tumor delineation, which is a key factor in brain tumor surgery planning (125, 126).

In situations where *in vivo* does not allow evaluation of lactate concentration, *ex vivo* analysis of biopsy tissue may be an alternative approach. Great care must be taken in sample collection and handling to avoid anaerobic glucose metabolism (127). However, provided

that samples are collected, stored and prepared for analysis under controlled conditions, the concentration of lactate can be accurately determined *ex vivo* using HR MAS MRS (7). In prostate cancer, significant differences in lactate and alanine concentration between benign lesions and prostate cancer biopsies has been reported (128).

3.1.2 Regulation of glycolytic activity studied by ^{13}C MRS— ^{13}C MR spectroscopy is a powerful tool for studies of metabolic pathways. The natural abundance of ^{13}C is low (1.1%), which gives this technique low inherent sensitivity. However, this can be turned into an advantage by use of isotopically enriched substrates. The metabolism of ^{13}C -labeled substrates can be traced in both in cultured cells and *in vivo*, as the fate of the incorporated ^{13}C can be monitored over time with little interference from background signals. The large chemical shift range for carbon allows resolution and identification of metabolites throughout entire metabolic pathways.

The abnormal glucose metabolism in cancer has been studied by ^{13}C MRS for more than two decades (120). For studies of glucose uptake, glycolytic flux and lactate formation, $[1-^{13}\text{C}]$ glucose or $[1,6-^{13}\text{C}]$ glucose is typically used. These isomers allow evaluation of the uptake and degradation of glucose, as well as the formation of metabolic intermediates and ultimately the formation and elimination of $[3-^{13}\text{C}]$ lactate. The rate of glycolysis can be quantitatively estimated through compartmental modeling, which is advantageous in studies of drug efficacy (129).

Initial studies in this field verified high flux through glycolysis in cancer cells (120, 130-133). *In vivo* investigation of the effect of tumor hemodynamics, hypoxia and substrate supply, has confirmed that high lactate concentrations principally are associated with aerobic glycolysis, not inadequate perfusion (134). It has also repeatedly been demonstrated that the rate of glycolysis can be modulated through cytotoxic drugs or, in the case of breast cancer, endocrine treatment (135-137). The effect of direct glycolytic inhibitors has also been investigated using ^{13}C MRS. Through dynamic studies of both cultured cells and culture medium, the kinetics of glucose metabolism can be studied, including the rates of glucose uptake and lactate secretion (138). ^{13}C MRS of tumors has the advantage of allowing longitudinal studies of glucose metabolism *in vivo*. In some applications, however, limitations in sensitivity or quantitative performance suggest the use of other methods. Glucose metabolism in tumors may in such cases be evaluated *ex vivo* using ^{13}C HR MAS MRS. After administration of ^{13}C -enriched tracers to the tumor-bearing animals and subsequent harvesting of tumor tissue, samples are available for immunohistochemistry or gene expression analyses after acquiring the ^{13}C spectra (139).

Overall, ^{13}C MRS is a versatile technique for evaluating the effect of drugs on glucose metabolism in cancer. Targeting the metabolic abnormalities of cancer with new drug entities is likely to benefit from ^{13}C MR spectroscopy as it allows noninvasive monitoring of surrogate biomarkers for therapeutic effect.

The low sensitivity constitutes a problem for clinical use of ^{13}C MRS. However, through optimized MRS protocols and injection of $[1-^{13}\text{C}]$ glucose, it has been shown that the kinetics of lactate formation in brain cancer can be investigated (140). Compared to ^1H MRS of brain cancer, this approach may be advantageous as it allows assessment of lactate formation rather than just presence of lactate in tumor tissue. The rate of lactate formation reflects the glycolytic capacity of the cancer cells, and are therefore suggested to be a better biomarker for grading brain tumors (134). In order to minimize the dose of $[1-^{13}\text{C}]$ glucose needed, polarization transfer pulse sequences and high field strengths (3T and above) are preferential. Although the method described by Wijnen *et al* suffers from relatively low

spatial (50 cm³) and temporal (2.3 min) resolution, it can be used to demonstrate that the rate of lactate formation in tumor tissue is higher than in normal brain tissue.

The potential clinical value of ¹³C MRS has also been demonstrated *ex vivo* in biopsies from prostate, lung and colon cancer. In natural abundance spectra, cancer-specific changes in various metabolites were found, allowing differentiation of tumor tissue from that of adjacent non-malignant tissue (141). Although similar data may be obtained more conveniently using proton MRS, the amount of information in ¹³C spectra could theoretically contain information that is not available in proton spectra. When applying multivariate analysis methods to metabolite patterns, ¹³C MRS data could potentially add statistical power and be used either alone or in combination with proton MRS data to resolve subtle differences between tissues.

3.1.3 Hyperpolarized MRS—Through the introduction of dynamic nuclear polarization (DNP), the interest in studies of glucose metabolism using MRS has been greatly increased. This technique can increase the signal-to-noise ratio by more than 50,000-fold, using hyperpolarized ¹³C-labeled substrates (142, 143). At 1.5T, the polarization level of ¹³C at thermal equilibrium is as low as 1×10^{-6} . Using a relatively rapid hyperpolarization procedure, polarization levels of $>2 \times 10^{-1}$ can be achieved (143). The substrate to be hyperpolarized is present in the solid state and doped with a single-electron containing compound. At low temperatures (1K), the electrons can be highly polarized due to their high gyromagnetic ratio. In DNP, this polarization is transferred to coupled nuclear spins by microwave irradiation near the resonance frequency of the electrons. The hyperpolarization is retained when the material is rapidly heated and dissolved, producing an injectable liquid within few seconds.

Due to time constraints and specific properties of hyperpolarized material, dynamic MRSI of hyperpolarized substrates offers challenges, which typically are absent in spectroscopic imaging at thermal equilibrium. Firstly, the time window for imaging is restricted to two to three times the T1 relaxation time. This means that hyperpolarized ¹³C MRSI must be completed within a few minutes after preparation of the tracer. Secondly, the longitudinal magnetization that has been created in the hyperpolarization procedure is not recovering during imaging. Thus, in addition to the inevitable loss due to T1 relaxation, the RF pulses applied in imaging convert longitudinal magnetization to transverse magnetization. In order to minimize magnetization loss, two imaging strategies are employed. Through the use of rapid low flip-angle RF pulses, only a small fraction of longitudinal magnetization is destroyed by each pulse. In addition, as much signal as possible is acquired per excitation through the use of rephasing pulses (144). Another issue is that imaging metabolic conversion is associated with rapid changes in signal. In sequences that use multiple excitations, these changes may convolve into k-space and cause image artifacts (145). Due to the very low interference from background and the more than 50,000-fold increase in signal after DNP, the signal-to-noise ratio is very high in the initial phase of hyperpolarized ¹³C MRSI. As hyperpolarized ¹³C imaging primarily is restricted by time and not by signal strength, EPI-based readout has often been found suitable (146). By using metabolite-specific low flip-angle excitation pulses, adiabatic refocusing pulses and echo planar readout, hyperpolarized ¹³C MRSI is performed in small animals with voxel sizes in the range of 0.135 cm³ with a temporal resolution of around 5 seconds, as shown in an example of a preclinical prostate cancer model in Figure 5 (146, 147).

The first substrate to be proven useful in characterization of cancer was [1-¹³C] pyruvate (148). For both physiochemical and biochemical reasons, this is an attractive substrate for ¹³C enrichment. Biologically, pyruvate is the end product of glycolysis and a starting point for the TCA cycle, lactate production and alanine production. Among its desirable

physiochemical properties is its compatibility with the DNP hyperpolarization method. Pyruvate forms an amorphous solid at low temperatures, which, after hyperpolarization can be readily dissolved in a suitable heated buffer (144). In addition, the signal from the C-1 carbon of both pyruvate, lactate and alanine has a long T1 (approximately 45 seconds in blood) (149). Thus, longitudinal magnetization of [1-¹³C] pyruvate is maintained for a clinically relevant period of time, allowing spectroscopic imaging of pyruvate uptake and metabolism in distal tissues. In humans, intravenously injected substances reach all major organs within approximately 15-40 seconds (144). Pyruvate is rapidly taken up and metabolized by most organs. After uptake, the pyruvate metabolite pattern is dependent on the nature of the tissue. In tissues where [1-¹³C] pyruvate predominantly is feeding into the TCA cycle, it is decarboxylated, producing acetyl-CoA. The resulting generation of H¹³CO₃⁻ can be assessed in real-time using MRS (150). In muscle tissue, transamination of [1-¹³C] pyruvate leads to formation of [1-¹³C] alanine, whereas its predominant metabolic fate in cancer is reduction to [1-¹³C] lactate (148, 151). The rate of [1-¹³C] lactate formation depends on LDH expression and the endogenous pyruvate and lactate concentrations, and application of dynamic modeling has suggested that the rate of conversion may be associated with tumor grade (152, 153). The conversion rate is dose-dependent, which must be taken into account during interpretation of MRSI data following [1-¹³C] pyruvate injection (153). The blood-brain barrier (BBB) may be a limiting factor for pyruvate uptake in healthy brain tissue within the time frame of hyperpolarized MRSI. However, in xenografted gliomas, it has been shown that uptake and lactate production differs significantly from normal tissue (154). This is assumed to be an effect of disrupted BBB in tumor tissue.

Primarily, [1-¹³C] pyruvate has been shown to be useful in distinguishing tumor tissue from healthy surrounding tissue, both in prostate cancer, brain cancer and sarcoma. However, as treatment with cytotoxic drugs may reduce the glycolytic rate in cancer, hyperpolarized [1-¹³C] pyruvate could also potentially be used in therapy monitoring. This was first shown in a solid murine lymphoma model, where the rate of [1-¹³C] lactate formation and the LDH activity was significantly decreased after treatment with etoposide (155). The potential value of hyperpolarised ¹³C MRS in monitoring the effect of drugs targeting metabolic pathway has recently been demonstrated by Ward *et al*, who showed that inhibition of PI3K by LY294002 or everolimus caused a decrease in [1-¹³C] lactate formation both in glioblastoma (*in vitro* and *in vivo*) and breast cancer (*in vitro*) (156). This reduction was associated with decreased HIF-1 expression and LDH activity, in accordance with downstream effects of PI3K inhibition.

Although [1-¹³C] pyruvate is well suited for studies of glucose metabolism in cancer, several other substrates have also been suggested. One example is [2-¹³C] fructose, which can reach sufficient polarization levels through DNP and has a T1 which allows hyperpolarised MRSI studies. Fructose has an early entry into glycolysis through the action of hexokinase, yielding fructose-6-phosphate. In a transgenic mouse model of prostate cancer, it was shown that hyperpolarized [2-¹³C] fructose was taken up by both benign and malignant tissue, but that significant conversion to [2-¹³C] fructose-6-phosphate only occurred in voxels containing malignant tissue (157). Another potential marker of tumor response to therapy is [1,4-¹³C] fumarate. In a murine lymphoma model, the production of [1,4-¹³C] malate was significantly increased 24 hr after administration of etoposide (158). This change correlated with loss of plasma membrane integrity, suggesting the use of [1,4-¹³C] fumarate for early detection of cell death following chemotherapy.

For concurrent assessment of different metabolic pathways and tumor microenvironment, several substrates may be hyperpolarized, injected and imaged at the same time. Simultaneous hyperpolarization of [1-¹³C] pyruvate, H¹³CO₃⁻ (allowing tumor pH

measurements), [1,4-¹³C] fumarate and ¹³C urea (allowing evaluation of tumor perfusion) has been reported, providing a multimodal approach to evaluation of tumor aggressivity and response to therapy (159).

In summary, labeling and hyperpolarizing endogenous or exogenous substrates with ¹³C in specified locations allow downstream tracing of metabolic within clinically relevant timeframes. The polarization is retained through serial metabolic conversions, and the ability to image metabolic fluxes is in principle limited only by the longitudinal relaxation (160). This technology is rapidly developing and holds the potential to revolutionize the use of MRSI in molecular imaging of cancer. The method has been applied to a wide range of preclinical applications, and clinical studies have recently been initiated. Due to the combination of endogenous tracers, high sensitivity, non-invasiveness and absence of ionizing radiation, it is expected that hyperpolarized MRSI will be a valuable supplement to existing imaging modalities in the management of cancer.

3.2 Altered enzymes in glycolysis and tricarboxylic acid cycle (TCA) of cancers

The altered glucose metabolism in tumors involves several different processes. Firstly, cancer cells exhibit enhanced glucose uptake through upregulation of facilitative glucose transporters, primarily GLUT1. Overexpression of glucose transporters has been shown in most cancers (161). It has also been demonstrated that glucose transport rates are higher in cancer cell lines than normal cell lines even at the same rates of proliferation, suggesting an association between malignant transformation and increased glucose consumption (162).

Several enzymes in glycolysis have also been shown to be generally upregulated in cancer (163). Both the rate-limiting enzymes 6-phosphofructokinase (PFK1) and pyruvate kinase (PK) as well as concentration-dependant enzymes such as hexokinase 2 (HK2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), triose-phosphate isomerase (TPI), phosphoglycerate kinase 1 (PGK1) and enolase 1 (ENO1) are upregulated, allowing a sustainable high glycolytic flux.

Increased lactate production is a prerequisite for sustaining a high glycolytic flux. Conversion of pyruvate regenerates NAD⁺ from NADH, avoiding the depletion that otherwise would occur as a result of glycolytic NAD⁺ consumption. This is facilitated through upregulation of lactate dehydrogenase A (LDHA). Thus, the net effect of aerobic glycolysis in cancer cells is the formation of large amounts of lactate, which is present inside the cells in abnormally high concentrations and secreted through the action of MCT4, a monocarboxylic acid transporter, which also is upregulated in cancer (164, 165).

3.2.1 Role of the TCA cycle in cancer—Although cancer cells exhibit a significant increase in lactate production from pyruvate, their switch to aerobic glycolysis is not complete. A small fraction of their pyruvate is metabolized via the mitochondrial tricarboxylic acid (TCA) cycle (166). This generates some ATP, but more importantly also supplies the cancer cells with precursor molecules for pathways that consume TCA cycle intermediates. Cancer cells also typically use glutamine as a carbon source for anaplerosis. Using ¹³C MRS, it has been shown that glutamine catabolism exceeds the nitrogen requirements for nucleotide production. Instead, glutamine provides a supplementary carbon source allowing tumor cells to use glucose-derived carbon and TCA cycle intermediates for biosynthesis of fatty acids and other macromolecules (166). Although the flux through the TCA cycle is relatively smaller than the flux through glycolysis in many cancers, their TCA cycle is functionally intact and the enzymes of the TCA cycle are not generally dysregulated in cancer. Interesting exceptions are succinate dehydrogenase and fumarate hydratase, which have been established as tumor suppressors (167). Mutations in these genes have been shown to cause certain cancers such as paraganglioma, pheochromocytomas and renal cell

carcinoma (168-170). Intriguingly, these cancers conform to Otto Warburg's original hypothesis that mitochondrial defects are a root cause of cancer.

3.3 Oncogenes and signaling pathways arriving at cancer-phenotypic endpoints of glucose metabolism

The increased glycolysis in cancer is associated with different regulation mechanisms, which are displayed in Figure 4. Firstly, glycolytic enzymes are induced by abnormal activity in several different groups of oncogenes. Secondly, stabilization of HIF-1 may also contribute to this enzyme induction. The relative role of these two systems are not completely clear, but it should be noted that cancer cells which do not experience hypoxia, such as leukemic cells which reside in the blood stream under high oxygen tension, may be highly glycolytic (115). Thus, HIF-1 may not itself contribute to the switch from normal metabolism to extensive aerobic glycolysis, but is rather a mechanism for sustaining high glycolytic flux and cellular proliferation rate under hypoxic conditions.

3.3.1 PI3K/AKT/mTOR, c-Myc, and p53 as regulators of glycolytic activity—In normal cells, glycolysis is tightly controlled through feedback mechanisms such as inhibition of PFK-1 and PK by ATP. In cancer cells, a coordinated regulation of proteins controlling glycolysis is frequently brought about through the action of upstream signaling pathways. Most of the regulatory mechanisms that increase glucose metabolism in cancer cells involve mutations in the *PI3K/Akt* pathway, *Myc* or *p53*, all frequently occurring tumorigenic mutations in cancer.

Mutations that enhance PI3K signaling is a common feature of many cancer types, and amplification of its downstream effectors AKT and mammalian target of rapamycin (mTOR) regulates several cellular responses to growth factor stimulation, which in itself may be sufficient to switch the metabolism from mitochondrial respiration to aerobic glycolysis (115). AKT signaling induces expression of GLUT1 through the action of mammalian target of rapamycin (mTOR), and also activates HK2 association with mitochondria promoting phosphorylation of glucose (171, 172).

Another oncogenic transcription factor that has been associated with increased glycolytic flux is *c-Myc*. Increased *c-Myc* expression upregulates lactate dehydrogenase A (LDHA), which in turn converts pyruvate to lactate. It has also been shown that *c-Myc* directly regulates other genes in the glycolysis such as GLUT1, HK2, PFK1 and enolase 1 (ENO1) (173, 174).

A major tumor suppressor gene, *p53*, has also recently been shown to be involved in cancer cell glucose metabolism. Although mechanisms are not completely elucidated, mutations in this gene modulate the balance between oxidative phosphorylation and glycolysis through various transcription factors (175).

3.3.2 HIF-1 as regulator of glycolytic activity—Cancer cells are frequently exposed to decreased oxygen availability. The HIF-1 transcription factor complex is activated in hypoxia and coordinates several adaptive mechanisms. In order to reduce oxygen consumption requirements by reducing oxidative phosphorylation and increasing lactate production, HIF-1 stimulates LDHA activity and reduces pyruvate dehydrogenase-kinase 1 (PDK1) activity. In addition, HIF-1 stimulates expression of both glucose transporters and glycolytic enzymes (176). Several enzymes in the glycolysis are positively regulated by both HIF-1 and c-MYC, potentially making them attractive targets for therapeutic drugs (116).

In summary, mutations in major oncogenes and tumor suppressor genes stimulate glucose transport, increases flux through glycolysis and suppress the entry of pyruvate into the TCA

cycle. This abnormal regulation of glucose metabolism is specific for cancer cells, as they acquire a self-sustainable anabolic phenotype that is not depending on extrinsic growth factors.

3.4 Targeting of glucose metabolism for cancer therapy

It may seem contradictory for tumor cells to generate energy through lactate production when oxygen levels would allow oxidative phosphorylation. However, the above-mentioned changes in glycolytic activity all contribute to a significantly increased glycolytic flux in cancer cells. This means that aerobic glycolysis may provide adequate amounts of energy to cancer cells while also providing essential precursors for biosynthesis (115, 177). In addition, the cancer cells gain an increased probability of surviving periods of limited oxygen supply. Secretion of lactate may also play a role in blocking immune responses and aid in invasion of neighbouring tissue (178).

As the high level of aerobic glycolysis appears to be beneficial for the survival and proliferation of tumor cells, metabolic pathways have recently been recognized as attractive drug targets. One possible approach in this respect is the direct targeting of enzymes involved in glucose metabolism. Inhibition of glucose transport has negative effects on tumor growth *in vivo*, but has currently no clinical utility (179). Several hexokinase inhibitors have also entered clinical trials based on encouraging findings *in vitro* and *in vivo* (180-183). Glycolytic inhibitors have been shown to sensitize tumors to other chemotherapeutic agents, providing a new possible approach to treatment of chemoresistant cancers (184-186). Currently, their role in cancer management is unclear. Ongoing clinical studies will hopefully provide new insight in the role of this drug class.

It has become increasingly evident that multiple genetic and epigenetic alterations are a major mechanism in cancer development, and that these alterations in many cases contribute to altered glucose metabolism. This has resulted in development of drugs that target upstream regulation of metabolic pathways rather than effector enzymes. An example is imatinib, which inhibits the chronic myeloid leukemia-specific oncogene product BCR-ABL. Among other effects, imatinib decreases HK and G6PD activity, thereby suppressing aerobic glycolysis and depriving cells of metabolic intermediates (187). Currently, drugs targeting PI3K, mTOR, AKT and HIF-1 are in clinical trials, suggesting that inhibition of oncogenic signalling pathways may be an important approach to treatment of cancer in the near future (188).

Development of drugs that directly or indirectly inhibit the increased glucose metabolism is likely to benefit from noninvasive techniques, such as MRS, for assessment of drug efficacy. In preclinical drug development, MRS has been frequently used for studies of glucose metabolism kinetics in cell cultures and *in vivo* model systems (138, 156, 189, 190). An increasing number of studies suggest that MRS also may play a role in clinical cancer management through direct assessment of glucose metabolism (128, 140, 191). The current interest in targeting of oncogenic signalling pathways regulating cancer glucose metabolism combined with the developments in hyperpolarized ^{13}C MRSI for molecular imaging suggest an important role for use of MRS in cancer management in the future.

4. Conclusions

Versatile MRS techniques are currently already available to detect a multitude of metabolic information noninvasively *in vivo*, and in HR MRS applications *ex vivo*. The metabolic information provided by MRS can give important information on the molecular make-up of tumors, which is crucial in the ongoing transition of clinical cancer care towards personalized molecular medicine. The information contained in the MRS-detected

metabolome can be utilized to aid in the diagnosis of cancer, to help select appropriate anticancer therapies, to monitor the tumor response to anticancer treatments, and to develop and evaluate novel targeted molecular therapies. Particularly in the development of novel anticancer therapies that target enzymes in choline and glucose metabolism, MRS techniques are currently proving extremely valuable, with a high potential for clinical translation. Certain MRS-detected metabolites can also serve as biomarkers or surrogate markers for novel molecular therapies that target oncogenic signaling pathways that have a downstream effect on a given MRS-detected metabolite. MRS can easily be integrated with MRI applications that provide complementary information on tumor anatomy.

The limitations of MRS techniques lie in their relatively low detection sensitivity, which can result in insufficient spatial and spectral resolution for clinical cancer detection and treatment monitoring. However, the availability of ever increasing magnetic field strengths and inventions of novel techniques such as hyperpolarization are continuing to push the limits of currently available MRS techniques. With its unmatched ability to provide a multitude of molecular information, MRS applications will undoubtedly play an important role in the future of molecular and personalized cancer care.

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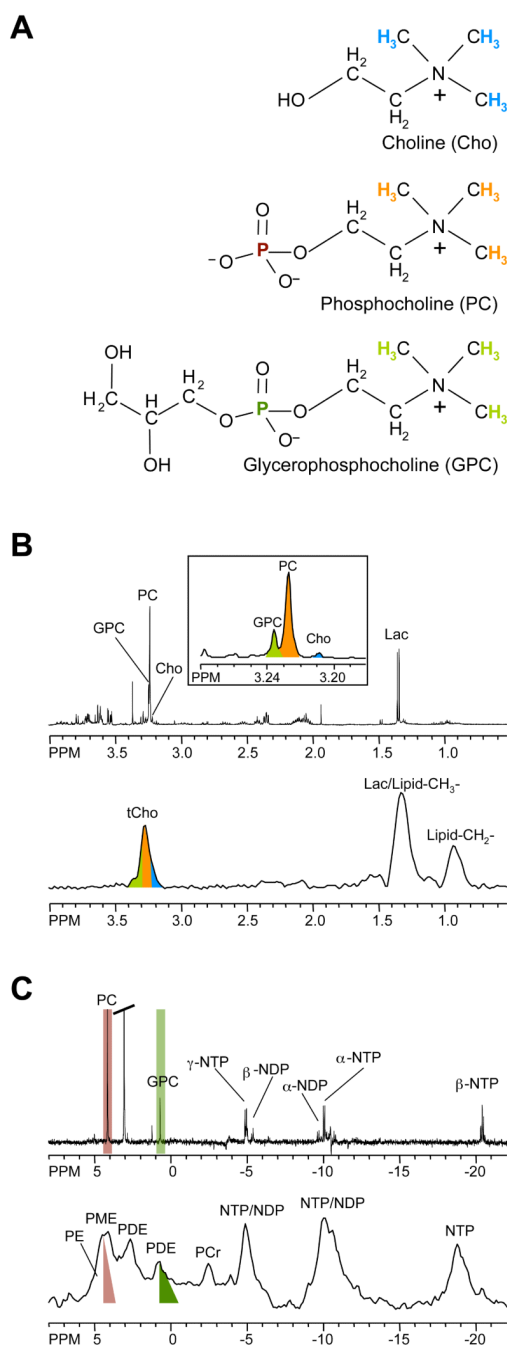


Figure 1.

(A) Chemical structures of the choline phospholipid metabolites free choline (Cho), phosphocholine (PC), and glycerophosphocholine (GPC). (B) High-resolution *ex vivo* ^1H MR spectra of triple-negative human MDA-MB-231 breast cancer cell extracts (top) and *in vivo* ^1H MR spectra of the same cell line grown as orthotopic tumor (bottom). (C) High-resolution *ex vivo* ^{31}P MR spectra of triple-negative human MDA-MB-231 breast cancer cell extracts (top) and *in vivo* ^{31}P MR spectra of the same cell line grown as orthotopic tumor (bottom). Assignments: Cho, free choline; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; DPDE, diphosphodiester; NDP, nucleoside diphosphate; NTP, nucleoside triphosphate; Lac, lactate; Lipid- CH_2 -, methylene groups of mobile lipids;

Lipid-CH₃-, methyl groups of mobile lipids; PC, phosphocholine; PE, phosphoethanolamine; PCr, phosphocreatine; Pi, inorganic phosphate; tCho, total choline-containing compounds (Cho+PC+GPC). The ¹H and ³¹P nuclei in Cho, PC, and GPC and their respective ¹H and ³¹P signals in the MR spectra are color-coded to identify the MR signals that arise from the corresponding nuclei.

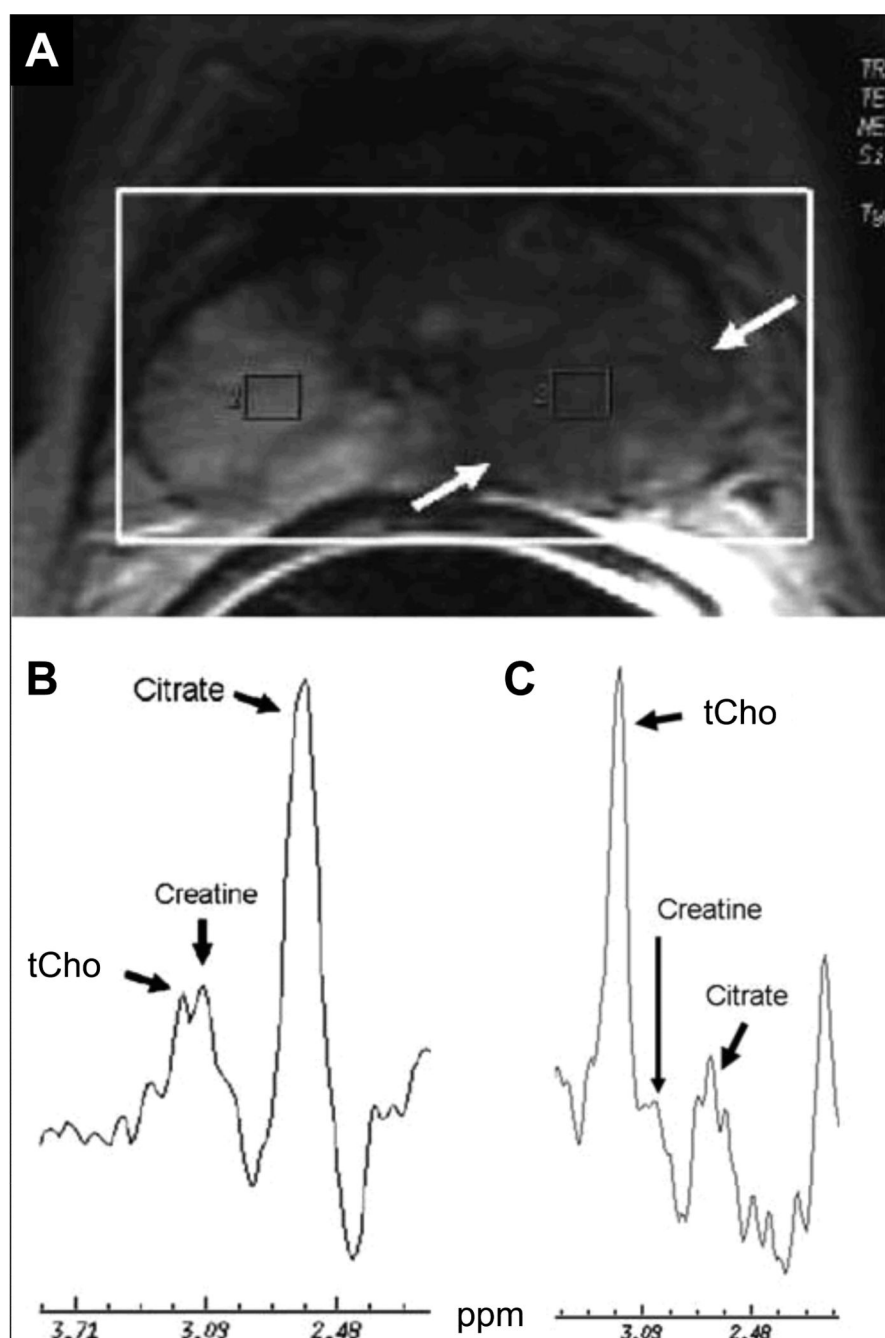


Figure 2.

Axial T2-weighted MR image and MRSI of the normal prostate gland and prostate cancer obtained at 1.5 T. (A) The MRSI was acquired from the whole gland (heavy white box) showing a large low signal intensity lesion (arrows) in the left peripheral gland. (B) The right voxel from the peripheral gland shows a high T2-weighted MRI signal intensity with the normal metabolic profile of prostatic gland, characterized by a high level of citrate and lower levels of choline and creatine. (C) The corresponding spectrum from the left voxel shows increased choline and reduced citrate, indicative of the metabolic profile of prostatic tumor combined with low signal intensity on T2-weighted MRI. Adapted from (33).

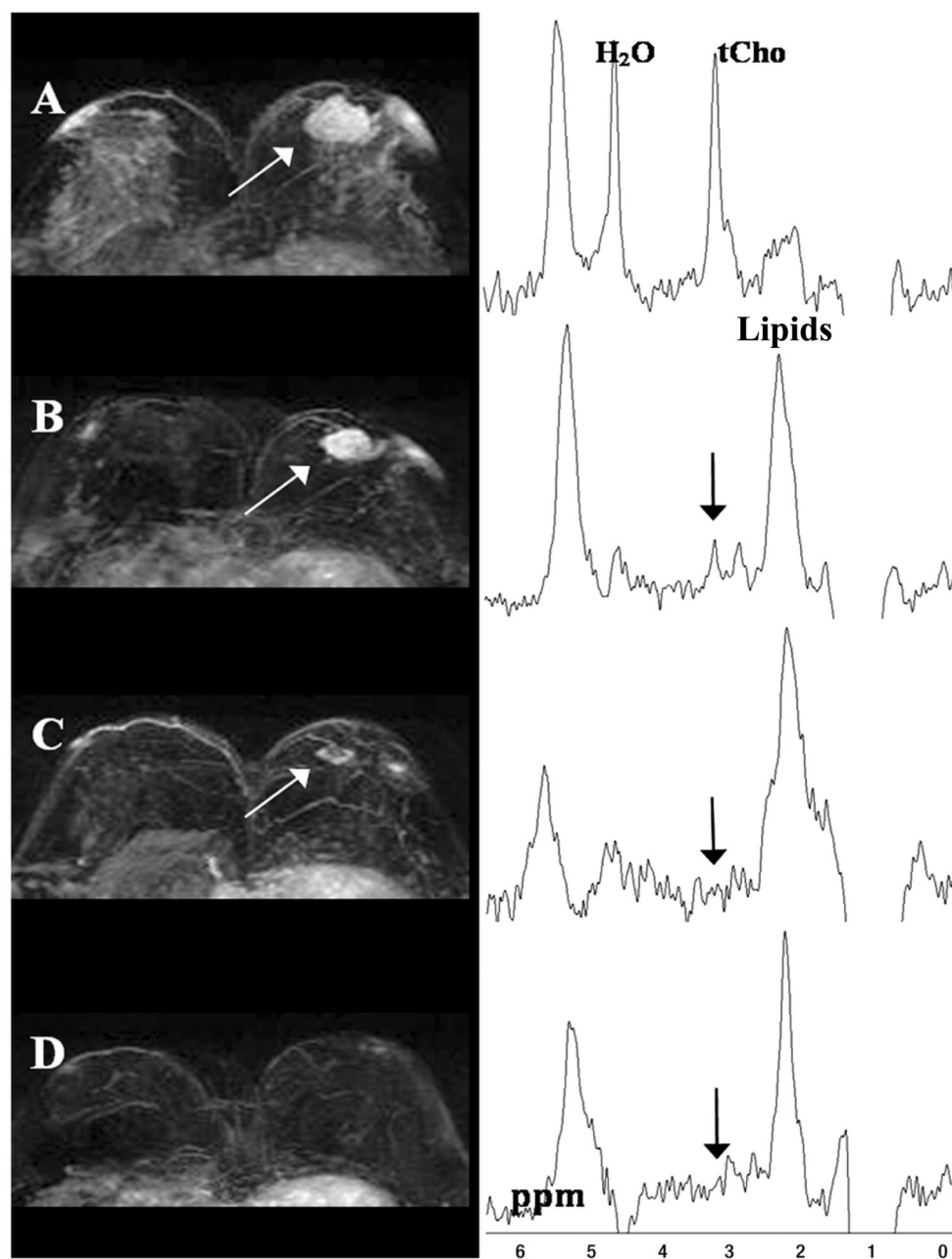


Figure 3. Maximum intensity projection MR images (left) and corresponding MR spectra (right) from a 40-year-old patient breast cancer patient who achieved pathological complete response. (A) Before treatment, a 3.4-cm lesion (arrow) shows a heterogeneous enhancing pattern. An elevated tCho peak (right) is visible at 3.23 ppm in the water-fat suppressed MR spectrum. Gaussian model fitting of the tCho peak gave 2.33 ± 0.54 mmol/kg tCho. (B) Enhancing lesion (white arrow) and water-fat suppressed spectrum (black arrow) acquired after one neoadjuvant chemotherapy (NACT) cyclophosphamide (AC) cycle. Lesion size was reduced to 2.6 cm, the tCho peak was visible at 3.22 ppm, and tCho = 1.15 ± 0.25 mmol/kg. The tCho level was reduced by 51%, while the tumor size decreased by 23%. (C) At second follow-up after two AC cycles and one taxane regimen, the lesion (white arrow) further

shrank to 1.3 cm, indicating good response. (D) After completion of all neoadjuvant chemotherapy cycles, the breast lesion completely regressed. The tCho peak (arrow) was no longer detectable (100% reduction) in the water-fat suppressed spectrum. Adapted from (22).

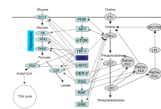


Figure 4.

Schematic showing the most important cancer-related cell signaling pathways (center) that impact upon enzymes in glucose metabolism (left) and enzymes in choline phospholipid metabolism (right). Arrows depict an activation in the direction of the arrow, and short vertical lines at the end of a line symbolize inhibition in the direction of vertical line.

Abbreviations: acetyl-CoA, acetyl coenzyme A; AKT, v-akt murine thymoma viral oncogene homolog 1; CCT, CTP:phosphocholine cytidyltransferase; Chk- α , choline kinase alpha; c-Myc, v-myc myelocytomatosis viral oncogene homolog; CPT, diacylglycerol; CT, choline transporter; CTP, cytidine-5'-triphosphate; Erk, extracellular signal-regulated kinases; GLUT, glucose transporter; GPC-PDE, glycerophosphocholine phosphodiesterase; HIF-1, hypoxia inducible factor 1; HK, hexokinase; LDH, lactate dehydrogenase; LPL, lysophospholipase; mTOR, mammalian target of rapamycin; PDH, pyruvate dehydrogenase; PFK1, phosphofructokinase 1; PI3K, phosphatidylinositol 3-kinase; PKM2, pyruvate kinase muscle isozyme 2; PtdCho-PLA₂, phosphatidylcholine-specific phospholipase A₂; PtdCho-PLC, phosphatidylcholine-specific phospholipase C; PtdCho-PLD, phosphatidylcholine-specific phospholipase D; TCA, tricarboxylic acid.

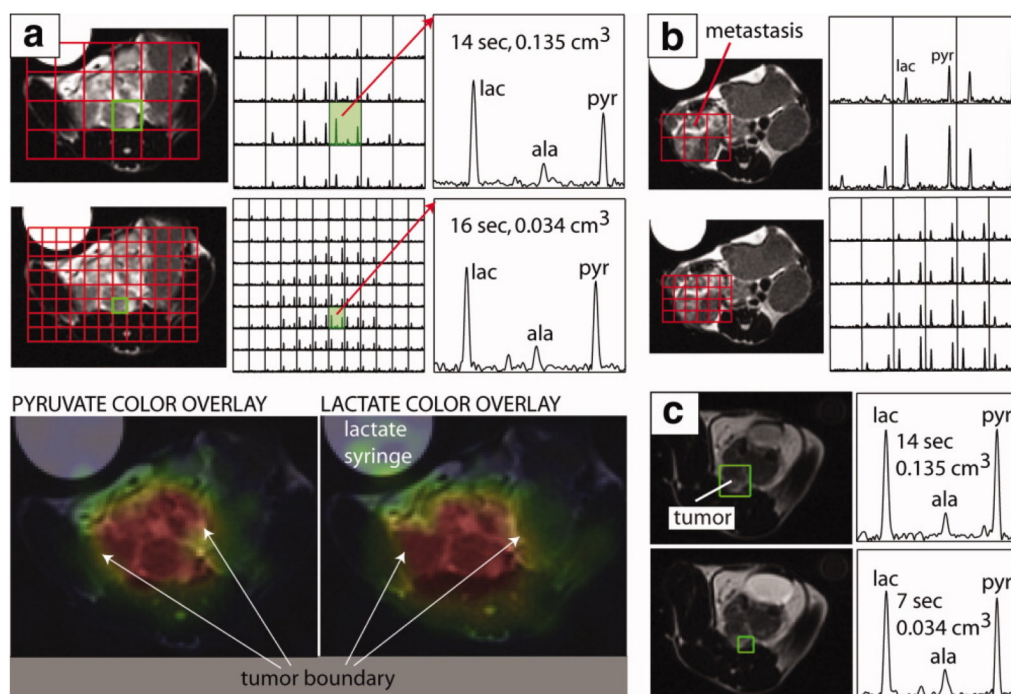


Figure 5.

In vivo MRSI of a transgenic murine mouse model of prostate cancer showing the effect of a compressed sensing imaging protocol. Pyruvate, lactate and alanine were detected in voxels down to 0.034 cm³ with TR=7s. The color overlay maps generated from the accelerated spectra show high intensity regions as brightly colored and highlight the spatial localization of metabolites according to tissue type. Tumor and non-tumor regions showed marked differences in metabolic profile. (a), (b) Comparison of spectra from un-accelerated (top panel) and accelerated (bottom panel) acquisitions with similar scan time demonstrating how increased spatial resolution can characterize metabolic heterogeneity and define tumor boundaries. (c) Comparison of spectra from un-accelerated and accelerated acquisitions demonstrating a 4-fold increase in spatial resolution and a 2-fold decrease in acquisition time. Figure adapted from (146) with permission.