Magnetic Resonance Metabolomics of Intact Tissue: A Biotechnological Tool in Cancer Diagnostics and Treatment Evaluation

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Abstract

Personalized medicine is increasingly important in cancer treatment for its role in staging and its potential to improve stratification of patients. Different types of molecules, genes, proteins, and metabolites are being extensively explored as potential biomarkers. This review discusses the major findings and potential of tissue metabolites determined by high-resolution magic angle spinning magnetic resonance spectroscopy for cancer detection, characterization, and treatment monitoring. Cancer Res; 70(17): 6692–6. ©2010 AACR.

The Concept of Metabolomics

The metabolome is the complete set of small-molecule metabolites in an organism and the final downstream product of the preceding gene expression and protein activity. Disease and influences from environmental factors, such as diet and drugs, are important factors in shaping the dynamic composition of the metabolome. The purpose of using metabolomics is to monitor the metabolic state at a certain time point and thereby understand more complex biological interactions, or to define biomarkers related to specific conditions. Compared with the more well-established "omics" technologies (genomics, transcriptomics, and proteomics), metabolomics is a relatively new and emerging field. Metabolomics contributes to the diversity of technologies within systems biology, thus enabling a more holistic picture of the chosen biological system.

Magnetic resonance spectroscopy (MRS) offers safe, nondestructive, and quantitative metabolite identification in an automated and high-throughput fashion. Its sensitivity is substantially lower than for mass spectrometry (microgram compared with picogram level), but MRS enables investigation of tissue samples by high-resolution magic angle spinning (HR-MAS) MRS with minimal sample preparation, keeping the sample intact after analysis (1). This review focuses on the use of HR-MAS in cancer detection, characterization, and treatment evaluation, in which several studies show that MR metabolomics has promise. Patients with identical clinical and morphologic cancer diagnosis may have very different outcomes. Moreover, the metabolic state in cancer compared with control tissue, or metabolic changes following response and/or resistance to therapy, usually involves multiple metabolites. Multivariate analysis, which handles interactions between multiple variables (metabolites), has, thus, become a commonly used strategy for analysis of large spectral data sets. Statistical methods, such as principal component analysis (PCA) and partial least squares regression (PLS), are then applied to matrices of spectral data for exploration and model building to establish classifiers that enable predictions and classifications related to the biological problem in question. Proper validation of the classifiers, preferably using separate data for training and testing, is of great importance. By this means, MR metabolomics can establish a more detailed tumor portrait by defining specific fingerprints reflecting diagnostic status or therapeutic response. Of vital importance is the identification of causal factors for these fingerprints, which may lead to the discovery of potentially useful biomarkers in a clinical context.

MR Metabolomics in Cancer Detection

To develop MR metabolomics as a clinical tool within oncology, basic differentiation between metabolic profiles of cancer and noninvolved tissue is fundamental. Such studies are also necessary for the general understanding of cancer development and progression. HR-MAS, in combination with multivariate analysis, clearly discriminates the metabolic profiles of cancer tissue and normal and/or adjacent tissue in different cancer types such as breast (2), colorectal (3), prostate (4), and cervical cancer (5). This differentiation is illustrated in Figure 1, where the score plot shows a complete separation of breast cancer and normal adjacent tissue. The most prominent differences are found in metabolites such as the choline-containing compounds (ChoCC), glycine and glucose. Multivariate analysis combines information from all metabolites detected in the tissue samples simultaneously. By interpreting these results, it is possible to identify single metabolites or ratios of metabolites that are the most
differently expressed. In this way, possible biomarkers can be detected and information about specific pathways obtained.

**Methyl donors and membrane building blocks**

ChoCC are important constituents in cell membranes, suggested to be markers for cell proliferation, and found elevated in a number of malignant lesions (3–10). In brain tumors, ChoCC levels are significantly increased compared with normal autopsy brain tissue (10). This finding was detected by measuring relative peak heights of phosphocholine (PC) and choline relative to creatine, resulting in ratios <6 for normal tissue, whereas brain tumor types such as meningiomas, schwannomas, and glioblastomas all gave ratios >200. Additionally, when comparing anaplastic ganglioglioma brain tumor to control tissue from epileptic surgeries, both choline and PC are significantly increased in the tumors (4.0 versus 0.6 µmol/g and 23.8 versus 6.8 µmol/g, respectively; ref. 9). In studies of ChoCC in prostate cancer, concentrations of PC and glycerophosphocholine (GPC) are significantly increased compared with normal tissues (0.39 versus 0.02 mmol/kg and 0.57 versus 0.29 mmol/kg, respectively), and when looking at the metabolic ratio of PC to GPC, it is significantly higher in prostate cancer tissue (3.5 versus 0.32; ref. 11). In breast, the ratio of GPC to choline is significantly
lower in cancer than in the noninvolved tissue from the same patients (0.75 versus 5.87 and 1.50 versus 2.55, respectively), whereas PC to choline is significantly higher in tumor samples (2.50 versus 0.69; refs. 6, 7). Changes in expression or activity of choline kinase and choline transporters may explain some of these differences. However, there is no joint biochemical understanding of the behavior of ChoCC in the different cancer types, which can be crucial for answering specific questions in tumor development and progression. Interesting future goals will be to determine whether there are grade-dependent changes in the ChoCC, or if there are differences between the metabolite ratios of all types of cancers, and also between invasive and preinvasive types of cancers in the various organs.

The ethanolamine-containing components (EthCC), glycerophosphoethanolamine (GPE), phosphoethanolamine (PE), and ethanolamine, are, like ChoCC, also major precursors and degradation products of membrane assembly and catabolism. In prostate cancer, PC to PE, PE to ethanolamine, and GPE to ethanolamine metabolic ratios measured by HR-MAS are significantly higher, compared with normal prostate tissue (0.08 versus 0.01, 16 versus 2.2, and 0.41 versus 0.06, respectively; ref. 11). PE concentration is also significantly higher in cancer tissue from cervical smears compared with normal tissue (6.76 versus 1.34 μmol/g; ref. 12). EthCC are interesting metabolites for cancer detection, and their concentrations are easily obtained by phosphorous MRS or two-dimensional total correlation spectroscopy.

Glucose metabolism and osmoregulation

Increased levels of lactate, and/or decreased levels of glucose, may indicate increased glycolytic flux or the Warburg effect and are observed in tissues of the brain (9), prostate (8, 13), and colorectal cancer (3). Absolute concentrations of lactate and alanine in prostate cancer biopsies measured by HR-MAS are significantly increased in cancer compared with benign tissue (1.59 versus 0.61 mmol/kg and 0.26 versus 0.14 mmol/kg, respectively; ref. 13), thus being potential cancer biomarkers also to be exploited in carbon hyperpolarized spectroscopic methods. Other metabolites such as myo-inositol, scyllo-inositol, and taurine are suggested to be important in balancing osmotic equilibrium and are increased in tissues of prostate (14) and colorectal cancer (3). Taurine levels are also significantly increased in brain tumors (16.0 in tumor versus 6.0 μmol/g in control; ref. 9).

Organ-specific metabolites

Citrate and polyamines are suggested to be biomarkers for prostate cancer. Healthy glandular prostate tissue is discriminated from cancer tissue by significantly higher levels of polyamines and the prostate-specific metabolite citrate (18.5 versus 5.28 mmol/kg and 43.1 versus 19.9 mmol/kg, respectively; ref. 8). Unfortunately, healthy stromal tissue is found to lack both citrate and polyamines, but it still shows lower levels of ChoCC than cancerous tissue. In brain tissue, N-acetyl aspartate (NAA) is a specific metabolite important for neuronal activity. NAA is seldom detected in tumor tissue because of low concentrations, and it is also used as an in vivo biomarker in brain diagnostics.

MR Metabolomics in Cancer Characterization

To explore the added clinical value of MR metabolomics, comparison of metabolic findings to clinical metadata is important. Relationships between the metabolic profile and factors such as tumor type, grade, lymph node status, hormone receptor status, and location in different neoplastic lesions have been investigated.

Tumor type

Survival rates among brain tumor patients can be significantly different depending on the tumor type and grade, and tools for more accurate diagnosis may affect the treatment decision and thus improve the clinical outcome. To distinguish brain tumor types (astrocytoma, glioblastoma, and meningioma), the inositol to creatine ratio is suggested as a possible biomarker (10). Higher levels of glutathione, glutamine, glutamate, PC, and PE are observed in the atypical meningioma compared with the benign type (15). Lipids dominate the HR-MAS spectra of brain metastases, but the aliphatic region of the spin-echo spectra has prominent signals of all commonly observed tumor metabolites (16). A relationship between the metabolic profile of the metastasis and the metastatic origin is suggested (16). This finding is of high clinical interest, as a patient may present with brain metastases with no previous knowledge about the primary tumor.

Tumor location

MR metabolomics have shown a unique metabolic phenotype related to the anatomic location of the colorectal cancer (CRC), which is clinically very important considering the recurrence rate and metastatic potential of these cancers (3). This is also the case for healthy gastrointestinal tissue, where each gut region generated a characteristic metabolic profile consistent with the varying structural and functional properties of the tissue at different longitudinal levels of the gut (17). Both of these studies indicate that HR MAS may be useful in analyzing local metabolic variation due to pathology in colorectal tissue biopsies.

Histopathologic grading

Determination of the histopathologic grade is an important part of cancer evaluation. Detection of cellular abnormalities preceding cancer gives useful information about the early biology of malignancy and may help define early predictors of cancer progression. Barrett’s esophagus might be associated with later development of malignancies. Normal squamous and Barrett’s epithelium are distinguished metabolically on the basis of choline and lipid-methyl to creatine ratios (1.99 versus 5.65 and 4.07 versus 7.4, respectively; ref.18). In smears from cervical cancer, concentrations of choline and PC are significantly higher in cancer than in moderate and severe dyskaryosis (1.00 versus 0.39 μmol/g and 2.71 versus 1.06 μmol/g, respectively; ref. 12). In the same study, levels of alanine and creatine are significantly reduced.
in normal tissue from cancer patients compared with normal tissue from noncancer patients (0.40 versus 0.65 μmol/g and 0.44 versus 0.97 μmol/g, respectively), implying that concurrent metabolite depletion occurs in normal tissue adjacent to the cancer tissue. When comparing metabolic profiles of brain tumors, alanine and valine, which are related to the anaerobic pathway, are increased in high grade (HGO) versus low grade oligodendrogliomas (LGO). Simultaneously, proline, glutamate, glutamine, gamma amino butyric acid, and NAA, which are related to the Krebs pathway, are decreased. This metabolic shift toward fermentative metabolism may indicate hypoxia in HGOs (19). In breast cancer patients without lymphatic spread, an important factor influencing further treatment decisions is breast cancer grade. Grade 2 and 3 invasive ductal carcinomas have been classified on the basis of the HR-MAS–determined metabolic profile (2). However, by comparing several multivariate techniques for this classification, large spreads in sensitivity and specificity are obtained, indicating low robustness of these results (2). Related to prostate cancer, much effort has been directed toward identification of metabolites descriptive for the Gleason grade. From a clinical point of view, this identification would be of great importance due to the difficulties in distinguishing the aggressive prostate cancers from those that are nonaggressive. So far, no robust metabolic markers for the prostate Gleason grade have been detected by MRS. However, spermine is proposed to be an endogenous marker of prostate cancer growth, because HR-MAS shows a correlation between the spermine concentration and the volume percentage of normal pros-taic epithelial cells, as measured by histopathology (20). Discrimination among different types and grades of CRC adenocarcinoma has also been attempted, but new studies with extended sample numbers will be necessary to obtain reliable correlations (3, 17). Determination of histopathologic grade is, to a certain extent, a subjective measure, and the results from different pathologists analyzing the same tissue sample may differ. It is, thus, important that studies relating metabolic profile to grade are carefully designed with this subjectivity in mind.

**Lymph node and hormonal status**

Axillary lymph node status is one of the most important prognostic factors in breast cancer. The same applies for the estrogen receptor (ER) and progesterone receptor (PgR) status, which, in addition, predict a possible endocrine responsive tumor. Biomarkers leading to the detection of lymph node metastasis early in the diagnostic progress are important for identification of high-risk patients and for establishment of personalized treatment. Increased taurine and reduced glycine are suggested to be major metabolic differences in lymph node–positive patients compared with lymph node–negative patients (6). To validate the predictive ability of these biomarkers, multivariate spectral classifiers were established to distinguish between lymph node–negative and –positive patients (2). Although the training validation indicated both sensitivity and specificity above 90%, the verification using blinded samples only predicted 8 of 12 patients correctly. Using a larger cohort, the classification was repeated (21), and the prediction accuracy obtained using 50 separate verification samples was approximately 68%. Although these results are better than random, this area is clearly in need of more thorough investigations. Various multivariate classifiers for hormonal status based on HR-MAS spectra of biopsies are established and further verified by tests using blind samples (2, 21). Using blind validation, a prediction accuracy of 88% (sensitivity 90%, specificity 82%) is obtained for ER status (21). ER-positive tumors contain more glycine, GPC, choline, and alanine, and less ascorbate, creatine, taurine, and PC when compared with ER-negative tumors. PgR-negative samples have more ascorbate, lactate, glycine, GPC, PC, choline, creatine, and alanine than PgR-positive tumors (21). MR-determined metabolic phenotype is suggested to have a future role as a supplement for clinical decision making about adjuvant treatment and the adaptation to more individualized treatment protocols, which may also be a valid suggestion for other cancer types.

**MR Metabolomics in Cancer Treatment and Monitoring**

The standard clinical cancer evaluation is not sufficient for accurate risk stratification or prediction of response to treatment, particularly with regard to evaluating the potential for drug resistance. Despite decades of research, the promise of personalized molecular medicine remains mostly unfulfilled. An exception is the treatment decisions routinely made on the basis of ER-, PgR-, and Her2-receptor status in breast cancer, whereas most other cancers lack such molecular measures. Development and validation of new risk stratification criteria based on tumor phenotypes, that is, metabolic profiles or fingerprints, for more robust differentiation, could lead to more efficient diagnostic and prognostic tools. Phenotypic differentiation of cancer will also contribute to increased understanding of critical pathways. This strategy provides a complement to gene expression–based profiling like that used to distinguish the five subtypes based on intrinsic genes in breast cancer (22).

Several studies are now in progress to detect new risk stratification criteria, markers for treatment response, and resistance in human cancers using MR metabolomics. To date, results mainly from animal studies and cell cultures have been published, of which some show promise for monitoring treatment response by HR-MAS. In a MCF7 xenograft model, a significant decrease in both choline and PC relative to creatine was detected 3 days after docetaxel treatment compared with control treatment (0.6 versus 0.95 and 2.9 versus 4.9, respectively; ref. 23), indicating lower proliferation due to treatment. Tumor apoptosis is another important marker of treatment response, and a robust biomarker for early detection of apoptosis will be of great value. HR-MAS has been used to explore the role of apoptosis in the development, progression, and treatment response of cervical cancers (24). The ratio of fatty acid methyl to methylene is associated with apoptosis in cervical cancer (24), whereas
taurine concentrations in gliomas correlate significantly with the apoptotic cell density, independently of the presence of necrosis (25). This findings suggest the benefit of using in vivo MRS measurements of taurine and fatty acids as markers for monitoring tumor apoptosis.

Prediction of survival for cancer patients is important in the choice of treatment strategy. The metabolic profiles of patients with brain metastases who survive more or less than 5 months are different when using PLS analysis (16), and the main metabolic differences indicated involve lactate, acetate, and glycine. In breast cancer, PCA indicates that high levels of taurine, GPC, and creatine, combined with low levels of glycine and PC, in the tissue specimen obtained during surgery characterize patients who remain healthy 5 years after surgery (26). Such predictive measures are evidently of clinical interest, but there is still a need for verification of the current results in larger patient populations, allowing for proper validation methods before such measures can become operative in a clinical setting.

Conclusion and Future Directives

Currently, there is a universal demand for personalized treatment, focusing on early identification of high-risk cancers for preventive treatment, instead of later progressive curative treatment. MR metabolomics of intact tissue samples is promising for obtaining a biological understanding of cancer disease, and may, eventually, extend the existing clinical tools for cancer diagnosis and treatment. The use of biomarkers for prognostic or predictive interpretation of a disease state has a long tradition in clinical medicine. We believe that MR metabolomics will be used for identification of multivariate biomarkers to aid in early detection of cancer, to stratify patients to receive the most efficient treatment, and to predict survival.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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