The personalized approach in cancer treatment stimulates the search for new analytical techniques, including spectroscopic methods such as Raman spectroscopy, mass spectrometry MALDI (matrix-assisted laser desorption/ionization) imaging and high-resolution magic angle spinning nuclear magnetic resonance (HR MAS NMR). The purpose of these studies is determination of metabolic profiles of cancer tissues, and their application in diagnostics and therapy of cancers. The review is mainly focused on application of HR MAS NMR technique. Qualitative and quantitative analysis of metabolites by means of this method is described for breast cancer tissues. In the near future HR MAS NMR in vitro studies of metabolic profiles combined with in vivo studies using MRI scanners may be applied as a new diagnostic tool.

Key words: tumor, metabolic profile, nuclear magnetic resonance, MALDI MS imaging, Raman spectroscopy.

Analysis of cancer tissues by means of spectroscopic methods

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Introduction

For effective treatment of cancers fast selection of therapy is essential. Responses to applied medicines can be different and personalized treatment requires collection of the maximum number of data [1]. One of them is the metabolic profile of the patient reflecting the present state of the organism. For this purpose spectroscopic techniques are applied, such as Raman spectroscopy, mass spectrometry MALDI imaging and high-resolution magic angle spinning nuclear magnetic resonance (HR MAS NMR). These techniques allow determination of the structure of chemical compounds and biomolecules, and are applied in cancer tissue examination. The purpose of these studies is identification of metabolites characteristic for cancer diseases and correlation of changes in metabolic profiles with extent of disease and probable prognosis.

Medical applications of spectroscopic methods

Raman spectroscopy is based on irradiation of a sample by means of laser and recording of scattered radiation. A significant difference was observed in the Raman spectra of benign and malignant tissues. For cancer tissues bands corresponding to C-C (1150 cm⁻¹) and C=C (1520 cm⁻¹) stretching vibrations of carotenoids and symmetric and asymmetric vibrations of the C-H at 2850–2940 cm⁻¹ for lipids were not observed; however, these bands were present in the spectra of non-cancerous tissues [2]. Diagnostic algorithms [3] were developed on the basis of Raman spectra tissues and tests of imaging were undertaken [4].

Mass spectrometry is based on ionization of the sample and separation of ions according to their mass to charge ratio (m/z). In analysis of tumor tissues the two-dimensional imaging of cells and tissues by means of MALDI imaging technique was performed. For prostate cancer two-dimensional maps of peptides and proteins containing 350-400 peaks within the range of m/z from 2,000 to 20,000 were recorded [5]. Two peptide ions, m/z 4,027 and 4,355, showed significant overexpression in prostate cancer in comparison with benign changes. The peptide ion at m/z 4,274 showed expression in benign tissues adjacent to epithelial and stromal cells while for prostate cancer it showed only a little or no expression. The peptide ion at 4,355 was identified as a fragment of mitogen-activated protein kinase – MEKK2 [5].

For breast cancer tissues there were detected six peaks in the range of m/z from 8345 to 8640, more intense in the presence of receptor HER-2. The peak at m/z 8404 was identified as cysteine-rich intestinal protein 1 (CRIPI), strongly associated with HER-2 overexpression. Application of MALDI imaging allowed prediction of HER-2 status with a sensitivity of 83%, a specificity of 92%, and an overall accuracy of 89% [6].

The protein profiles corresponding to early stages of gastrointestinal cancer disease were significantly different from advanced stage tumors. Signals
Basics of HR MAS NMR measurements

The NMR spectrum is a graph correlating signal intensity and the frequency of radiation absorbed by the atomic nucleus — in the case of tissues, proton atoms. Chemical shifts depend on the structure of the molecule and are expressed in ppm (parts per million). High resolution of the spectrum in HR MAS technique is obtained by spinning the sample around an axis inclined 54.7° to the direction of the static magnetic field [12, 13]. Spectra recorded in vivo by means of MRI scanners are of low resolution magic angle spinning NMR, which was introduced in 1996. The first publication on studies of tissues of lipoma and liposarcoma utilizing this method appeared in 1997 [9]. In 2009 two review articles about tissue spectra registration, identification of metabolites, determination of their concentration and relaxation times (T1 and T2) were published [10, 11].

Metabolic profiles of breast cancer determined by HR MAS NMR technique

Application of HR MAS NMR will be discussed in the example of breast cancer tissues because the most attention was focused on this issue. Cheng et al. were the first to study breast cancer tissues by means of this technique [25]. Reported spectra were dominated by signals from fatty acids. It was found that an increased ratio of lactate and choline concentrations is associated with an increase in tumor grade. It was shown that an increase of T2 relaxation time of phosphocholine can help to distinguish grade II and grade III of ductal carcinomas. Sitter et al. identified numerous metabolites: amino acids (mainly glycine, alanine, valine, leucine, lysine, isoleucine, tyrosine and histidine), metabolites associated with consumption of energy (lactate, glucose) and choline components (glycerophosphocholine and phosphocholine) [26].

Principal component analysis showed increased levels of lactate, creatine, glycine and phosphocholine from specimens with high numbers of tumor cells. Two groups of samples were studied: tumors smaller than two centimeters (T1) and bigger tumors (T2–T4). The last group of tumors was characterized by higher concentration of choline and glycine. Higher intensity ratios of glycerophosphocholine to phosphocholine and glycophosphocholine to choline were found for non-involved samples than for tumors, whereas the intensity ratio of phosphocholine to choline was lower for non-involved specimens [27].

On the basis of microarray studies, basal molecular subtype of breast cancer, an ERBB2-overexpressing group and two or three luminal subtypes A, B and C were distinguished. These subtypes indicated differences in gene expression profile and in prognosis and course of disease [28, 29].

Borgan et al. analyzed the data obtained from HR MAS with microarray studies of gene expression [30]. HR MAS and microarray analysis were performed for 46 samples of breast cancer. The quality of RNA obtained after HR MAS analysis for 11 tissues was insufficient; therefore for microarray analysis adjacent tissue from the same tumors was used. The examined tissues were diagnosed as invasive ductal carcinoma with ER receptor expression which belong to luminal A subgroup. Two strategies were applied to combine the gene expression and metabolic data: multivariate analysis to identify differ-
ent groups, and study of the correlation between levels of metabolites and biological processes. On the basis of gene expression 36 tumors were classified as luminal A subtype, one as basal, one as ERBB2, seven normal-like and one unclassified. All samples classified as luminal A were ER positive.

On the basis of HR MAS spectra 31 tissues were classified as luminal A subtype but within this subtype three subgroups were distinguished: A1, A2 and A3.

In subgroup A2 glucose and alanine concentrations were found to be significantly lower and higher, respectively.

**Fig. 1.** A – T2-weighted MRI image of prostate; B – MRSI spectrum taken from the voxel shown in A; C – 1H HR MAS NMR spectrum of excised tissue; D – histological picture of examined specimen, in 60% contain stromal tissue – H&E staining.


(From: Swanson M.G. et al., Magn Res Med 2003; 50: 944-954)
The intensity of α-hydrogen amino acid signals was significantly lower for A1 and higher for A3. Signals of lipid residues were significantly higher and signals of myo-inositol were significantly lower in A1 than in A2 and A3. Moreover, subgroup A2 was enriched for a gene related to the cell cycle and DNA repair. The A2 group’s metabolic and transcriptional features indicate a higher Warburg effect and proliferation compared with the other luminal A samples. In the second strategy the correlations between concentrations of myo-inositol, taurine, creatine, glycerophosphocholine, phosphocholine, glycine and choline and all transcripts in microarray were investigated [30].

Concentrations of the amino acids alanine, glycine, leucine, isoleucine, lysine, valine, histidine, tyrosine, glutamate, phenylalanine, taurine and creatine were determined in 80 samples of breast cancer from patients from the tódz region by the HR MAS NMR method [31]. Moreover, the presence of metabolites such as myo-inositol, scyllo-inositol, choline, phosphocholine, glycerophosphocholine, lactate, acetate and β-glucose was proven.

A significant increase in the concentrations of creatine, choline, phosphocholine and glycerophosphocholine was found in specimens with increased numbers of cancer cells. Significantly higher concentration of PC was found for patients with higher degree of malignancy. A similar insignificant tendency exists for glycerophosphocholine, phenylalanine and glucose. After the division of samples into three categories (G1, G2 and G3), differences in concentration of phosphocholine disappear and remain only as a strong trend. There was no significant correlation between concentration of phosphocholine and Ki 67 proliferation antigen; however, there is a very evident trend. Lower Cr and Cho concentrations were observed for tumors with metastases in axillary lymph nodes. Reduced amounts of GPC, myo-inositol, glutamate and acetate were found for tumors with estrogen receptor. Only the glutamate level was significantly lower for tumors with the progesterone receptor. Increased level of myo-inositol and glutamate accompanies the presence of keratin CK5/6 (tendency for scyllo-inositol and isoleucine). Higher concentrations of Cr, GPC, acetate and glutamate were found for tumors with protein cyclin E present. A similar tendency was observed for alanine, choline, taurine, glycine, myo-inositol, leucine, lysine and reverse for histidine.

There are no significant differences in metabolite concentrations depending on the triple-negative phenotype (tendency towards higher concentrations for phosphocholine and glutamate). Size of tumor, presence of HER2 receptor as well as overall survival do not correlate with any metabolite concentration [31].

Conclusions

Modern diagnostic methods enable more precise and faster diagnosis of patients with cancer. Application of spectroscopic methods extend the analytical capabilities, in particular nuclear magnetic resonance. It can be expected that the development of nuclear magnetic resonance scanners and higher accessibility of HR MAS NMR measurements would allow us to improve the diagnosis of cancers based on the metabolic profile.

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