HR MAS MRS for Cancer Metabolomics

Altered metabolism is now considered an emerging hallmark of cancer [1]. The rapid proliferation rate of cancer cells is associated with specific metabolic demands. The cells must adapt to a hostile microenvironment with restricted supply of nutrients and they need to convert nutrients into biomass while maintaining sufficient energy production [2]. The cancer-specific metabolic phenotype with increased glucose consumption and aerobic lactate production is well known. In addition, abnormal phospholipid metabolism and shunting of metabolites from glycolysis into the pentose phosphate pathway is also commonly associated with cancer [3, 4].

Abnormal metabolism in cancer is increasingly recognized as a potential target for treatment in itself, but also as a source for novel biomarkers that can be used in diagnosis, for stratification of patients to treatment and measurement of treatment response. MR spectroscopy (MRS) is a commonly used technique for studies of cancer metabolism. Compared to mass spectrometry, MRS has lower analytical sensitivity. However, a relatively large number of metabolites relevant for cancer metabolism can be measured simultaneously, and the method is by nature quantitative. In contrast to many other techniques, *in vitro* and *ex vivo* MRS findings can be translated to noninvasive *in vivo* applications. Traditionally, MRS-based studies of cancer metabolism have been performed on liquid solutions, such as cell or tissue extracts. This allows rapid acquisition of high-quality spectra, but the sample preparation is labor-intensive and the sample is consumed. Thus, obtaining spectra from intact tissue with a method that preserves the tissue for subsequent analyses would be an advantage. Chemical shift anisotropy, dipolar and quadrupolar interactions will cause broad spectral lines in solid samples analyzed by traditional high resolution (HR) MRS. This limitation can be overcome by applying HR magic angle spinning (MAS) MRS. More than 50 years ago, Andrew [5] and Lowe [6] demonstrated how rapid spinning of samples at an angle (54.7˚) to the external magnetic field eliminates line broadening. The nuclear dipole-dipole interaction averages to zero at the magic angle, while the chemical shift anisotropy and the quadrupolar interaction are partially averaged. Spectra acquired using HR MAS MRS have a spectral resolution close to that of extracts, and depending on the method for extraction, allow assignment and quantification of the same metabolites [7]. HR MAS MRS therefore offers significant advantages for studies of cancer metabolism, including simple sample preparation and, importantly, the opportunity to perform subsequent analyses of the intact tissue specimens after MR analysis for complementary information (i.e. immunohistochemistry or gene expression). Many studies of biopsies from human cancers have proven that the metabolic profile contains predictive and prognostic information[8-20], but the method is not yet routinely used in the clinic. However, recent installation of spectrometers in close proximity to surgical theaters might facilitate this development[21, 22].

To obtain robust and reproducible data based on HR MAS MRS, several precautions and considerations are necessary. This includes tissue sampling or excision, storage, sample preparation, MRS acquisition and the further data analysis. Standardization of all these aspects and the development of more automated routines will be important future tasks in order to accommodate high quality multi-center studies and analysis of larger biobank cohorts. This lecture will cover motivation and rationale for using HR MAS MRS in cancer studies, and also basic knowledge for how to start and perform such studies.

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