Characterization of Brain Tumors Using 1H-NMR Spectroscopy and Self-Organizing Maps: Relevance of Hydrophilic and Lipophilic Metabolites.

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Introduction

Pattern recognition techniques gain increasing attention in the interpretation of in vivo MRS spectra of brain lesions (1). Furthermore, detailed information from ex vivo tissue analysis is needed to understand the underlying metabolic changes and to correlate them with the in vivo results (2).

We used high-resolution 1H-NMR spectrocopy in combination with feature extraction and visualization methods based on k-nearest neighbor classifiers (kNN) and self-organizing maps (SOMs) (3-5) in order to select and to characterize metabolites relevant to the differenciation of three classes of brain tumors. With a dual extraction procedure both the lipophilic and hydrophilic tissue fractions were obtained.

Material & Methods

Human brain tumor specimens were taken from 28 patients undergoing scheduled surgery. The dissected samples belonged to three classes of brain tumors: AS (astrocytoma (7) and oligoastrocytoma (3) of grade II and III), MG (meningeoma (10), grade I) and GB (glioblastoma (8), grade IV). The tissues were homogenized in a mixture of chloroform/methanol/ bidest.water (1:1:1). After centrifugation, the separated water phase was lyophilized and the organic phase was evaporated in a stream of nitrogen. Water-soluble metabolites were measured in D2O (pH*=7), the lipids were dissolved in CDCl3/MeOD (2:1). 1H-NMR spectra were recorded on a Bruker DRX 600 MHz spectrometer (water phase: CPMG-sequence with water-presaturation; lipid phase: water-presaturation).

The spectra were phase- and baseline-corrected. After elimination of lactate and solvent signals, they were normalized to the sum of all integrals and divided into peak-fitted (not equidistant) segments. These segments were integrated numerically to form a vector of 610 components (water-soluble metabolites) and of 349 components (lipids) for further analysis.

In the first step these data sets of 610 (349) variables were reduced to variables (NMR signals) relevant to the classification using the kNN method. Important metabolites and combinations of these were identified in cross-validated runs and served as input parameters for the visualization with a self-organizing map. By this means we were able to separate different classes of brain tumors.

Results

The system identified the important parameters and combinations for the separation of the three classes AS (astrocytoma & oligoastrocytoma), GB (glioblastoma), MG (meningeoma). Also characteristic metabolite signals for each tumor class were found (see below). All classification accuracies [%] are average sensitivity values.

Separation of all three classes: Most important metabolite signals are the resonances assigned to taurine (3.42 ppm, 82 % and 3.43 ppm, 81 %) and myo-inositol (3.61 ppm, 77 %) for the water-soluble metabolites. For the lipids, the signals of cholesterol (0.89 ppm, 77 %), fatty acids (1.70 ppm, 72 %) and PUFA (5.36 ppm, 69 %) were identified. Combinations of metabolites gave higher classification results with the water-soluble metabolites (4 signals (taurine, aspartate, acetate, GSSG), 97 %) being more characteristic than the lipids (3 signals, 78 %).

Separation of individual tumor types: Characteristic signals were those of myo-inositol (3.61 ppm, 93 %) for AS, phosphoethanolamine (PE, 3.98, 84 %) and fatty acids (lipids, 1.70 ppm, 88 %) for the glioblastoma, and aminoacids (3.73 ppm, 91 %) for the meningeoma. Combinations of 2 metabolites yielded classification accuracies of at least 94 %.

The figure depicts the contribution of selected metabolites in the process of classification: Astrocytoma (i.e. astrocytoma and oligoastrocytoma) are marked by the highest content of myo-inositol and of a lipophilic metabolite U1. U1 belongs to a group of oxidized poly-unsaturated fatty acids. Vice versa glutamate concentration is lowest in this group. Meningeoma show the highest amount of taurine, phosphoethanolamine, fatty acids and amino acids and the lowest concentration regarding cholesterol. Glioblastoma are characterized by an increasing cholesterol content with grade of malignancy within the group of astrocytic tumors (AS+GB).

The metabolic alterations shown on the SOMs confirm several results published by other groups (phosphoethanolamine, myo-inositol, glutamate), but reveal also differing or new results (U1, aminoacids).

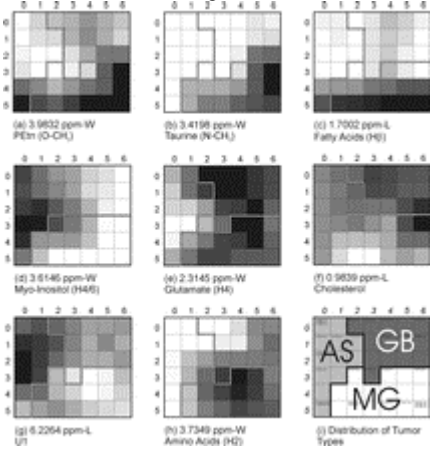

Figure: Self-organizing map visualization of relevant metabolites to differentiate three classes of brain tumors: AS (astrocytoma & oligoastrocytoma), GB (glioblastoma), MG (meningeoma). (i) shows a generalized SOM representation of the three classes (tumor types) separated by decision boundaries. (a)-(h) reflect the correlation of signal integrals with class locations (dark areas indicate high relative concentrations). `W´ and `L´ mark spectra of the water- resp. lipid-phase. For example (b) indicates that high taurine signals in the spectra of water-soluble metabolites are typical of the meningeoma tissue samples.

Classification with signals of water-soluble metabolites yielded higher average sensitivities than with lipid signals. This might be due to a higher steady state turnover in the metabolism of the water-soluble intermediates. Another cause might be tissue heterogeneity.

Until now, differences in the group of astrocytoma and oligoastrocytoma regarding tumor type and grade could not be identified due to the limited number of samples.

In our comparatively small sample pool, we found clear inter-class differences with good classification results. In order to minimize the danger of random artefacts our results should be evaluated in larger studies.

Conclusion

Characteristic metabolic profiles of brain tumors can be gained using a tissue dualextraction technique, high-resolution 1H-NMR spectroscopy and pattern recognition methods like k-nearest-neighbor and supervised self-organizing maps. By this means, astrocytoma, meningeoma and glioblastoma were classified with high accuracy using a combination of NMR signals in cross-validated classification runs. Characteristic metabolites for each group of tumor were identified and the alterations of concentration visualized in a clear and understandable form by SOMs.

References

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