Lipid profile of distinct areas of astrocytic brain tumors

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Introduction:

Astrocytic brain tumors – especially Gliomas – are characterized by heterogeneous tissue with no distinct transition to healthy tissue because of their infiltrating growth. In addition, tumour cells reveal an altered metabolism and tend to affect neighbouring healthy cells. This can be studied with high resolution magnetic resonance spectroscopy, as spectra of intact tissue or tissue extracts reflect numerous metabolic information. Lipids act not only as membrane components but also as signalling molecules, a.o. diacylglycerides (DAG). Furthermore, the fatty acid residues of phospholipids determine the fluidity of bio membranes. Therefore, we investigated changes within the lipid profile of distinct localisations of high grade astrocytic tumors (grade III and IV): viable, active tumor core (24 biopsies), tumor margin (18 biopsies) and more distant tumor margin (4 biopsies). In order to get detailed insight in the metabolism 14 lipid extracts were further separated into different lipid classes with a solid phase extraction method (SPE): free fatty acids, polar lipids and neutral lipids which were further separated in cholesteryl esters (CE), cholesterol (C), tri-, di- and monoacylglycerides (TAG, DAG, MAG).

Methods:

Tissue samples were extracted using a dual phase extraction technique with chloroform, methanol and bidistilled water. Extracts were dried under nitrogen, re-dissolved in 0.6 ml deuterated chloroform-methanol (2:1) and were analyzed with a Bruker Avance DRX-600 at 300K using a 5 mm H,C,N inverse probe with actively shielded gradient coils. Signal assignments of ^{1}H-NMR spectra were confirmed using heteronuclear 2D-NMR spectra. Eight lipid extracts of tumor core, four tumor margin extracts and two extracts of reference tissue were separated by means of SPE with aminopropyl and silica columns using a modified method after Kaluzny et al.[1]. The obtained SPE-fractions were prepared and analyzed as described above.

Results:

Lipid extracts from the different tumour sections revealed distinct lipid compositions: all extracts contained cholesterol, phospholipids and fatty acids in NMR detectable amounts. However, TAG, DAG as well as CE were detected only in extracts of the tumor core and tumor margin. These neutral lipids were absent in the more distant tissue. Furthermore, cholesteryl esters were identified in nearly all lipid spectra of the tumour core (21 of 24), while TAG appeared preferentially in Glioblastoma core extracts. However, dolichol seems to represent a metabolic marker of tumour cells, as it is detected in 22 of 24 tumor core extracts and in 11 of 18 extracts of the tumor margin indicating a higher amount of neoplastic cells in these biopsies, but it was absent in the distant tumor margin.
Also polar lipids like glycerophosphocholines and –ethanolamines as well as plasmalogens, sphingomyelins (SPH) and galactosyl cerebrosides (GS) occurred in varying concentrations in the different tissue sections: tumour core tissue extracts contained significantly higher amounts of glycerophospholipids (in particular phosphocholine), while plasmalogens were significantly lower concentrated in tumour core as well as tumour margin tissue compared to extracts of more distant biopsies (see fig. 1). Higher amounts of polyunsaturated fatty acids (PUFA) were detected in the tumour core: CE and TAG contained higher amounts of PUFA, while phospholipids contained higher amounts of omega-3 fatty acids and arachidonic acid within the SPE fractions of lipid extracts. The ratio of GS to SPH represents a discriminator also: it is very high in the distant tissue (3.33±0.22), but rather low within the tumour core (0.44±0.31). In the tumour margin a ratio of 1.35±0.38 is determined, which reflects the presence of malignant cells.

Conclusion:

Analysis of lipid profiles of tumour core, tumour margin and more distant tumour margin (i.e. likely unaffected tissue) gives evidence that extracts of the tumour margin contain characteristics of tumour core as well as distant margin resembling the ratio of tumor cells to healthy cells. Several metabolic markers were identified: CE and TAG are indicators of malignancy designating higher angiogenetic proliferation and higher amounts of lipid droplets. In addition, TAG seem to be specific for grade IV tumors. Also dolichol is characteristic for tumour cells, as the dolichol concentration is low in healthy brain and therefore not detectable in the distant tumour extracts. Furthermore, the ratio of galactosyl cerebrosides to sphingomyelin represents a discriminator. Finally, higher amounts of PUFA of the phospholipids indicate an increased request for membrane fluidity and could indicate disordered signalling pathways, i.e. increased formation of eicosanoids from arachidonic acid.

References:

[1] M.A. Kaluzny et al., *Journal of Lipid Research* 1985; Vol. 26; 135-140.

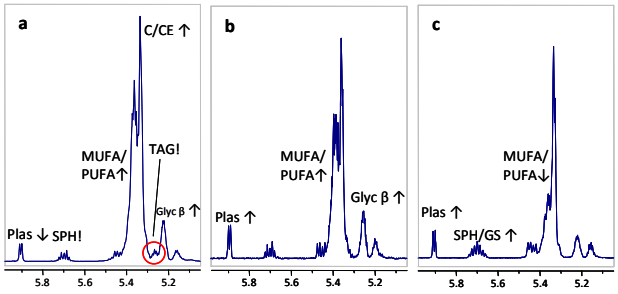

Fig. 1: ^{1}H-NMR-sections of the low field part of lipophilic extracts obtained from a) tumor core (GBM IV) b) tumor margin and c) more distant margin. Characteristic changes are emphasized.

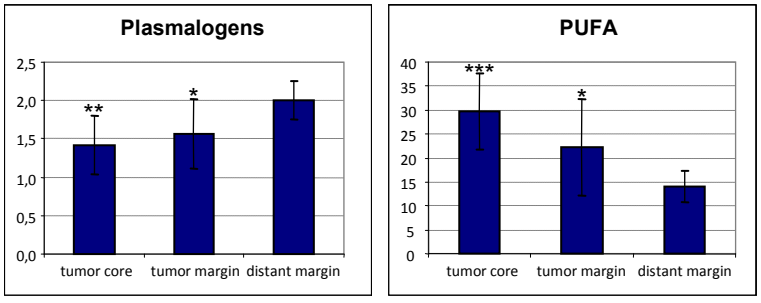

Fig. 2: Integral values of plasmalogens and PUFA in tumor core, tumor margin and more distant margin. Significant differences to distant margin were marked as p<0.05 (*); p<0.01(**), p<0.001(***).