# Identification of lipids in tissue extracts of astrocytic brain tumors

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## Introduction:

Prerequisite for accurate resections of human brain tumors is the differentiation between tissue of tumor core and tumor margin. All tumor tissue needs to be removed to avoid tumor relaps and to enhance prognosis, but healthy tissue should not be resected coevally in order to maintain normal brain functions. Therefore, the amount of tumor cells and healthy cells in the margin of the tumor is crucial in order to decide about a resection of this tissue. Different tissue types show distinct metabolite profiles, which can be observed with magnetic resonance spectroscopy. High resolution spectra of intact tissue or tissue extracts reflect numerous metabolic information and analysis with pattern recognition algorithms enables tumor classification.[1,2] If the metabolite profiles of tumor margins are different enough, they can be distinguished from tumor cores.
Therefore, 20 lipophilic tissue extracts of tumor core and tumor margin (each with ten samples) were analyzed with NMR spectroscopy.

## Methods:

Tissue samples were extracted by using a dual extraction technique with chloroform, methanol and bidistilled water. The organic extracts were dried under nitrogen, weighed and redissolved in 0.6 ml deuterated chloroform-methanol (2:1). The lipophilic components 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 are confirmed using heteronuclear 2D-NMR spectra. Solid phase extraction with aminopropyl columns of a lipid extract of a tumor core tissue was accomplished using a method after Kaluzny et al[3]. The obtained fractions of the SPE were also dried under nitrogen and redissolved after evaporation in 0.6 ml deuterated chloroform-methanol (2:1). 1D $^1$H-NMR spectra of the SPE fractions were aquired as well as various homo- and heteronuclear 2D-NMR spectra.

## Results

The lipophilic tissue extracts showed various lipids for example different fatty acids, neutral lipids as well as polar lipids. Furthermore, we observed olefinic protons in HSQC-spectra, which appear upfield of signals from unsaturated fatty acids and to our knowledge not been detected in tumorous tissue before. The respective signal in the $^1$H-NMR spectrum is shown in figure 1. These resonances were identified in 9 of 10 $^1$H-NMR spectra of tumor cores, but they were not absent in spectra of the tumor margins – with one exception. Different 2D-NMR spectra were recorded for structure elucidation, which identify an olefinic molecular substructure (see fig.2), but a complete structure elucidation couldn´t be achieved within the complex tissue extract spectra due to a large signal overlap. In order to simplify the structure elucidation the lipophilic extract was separated into different classes by a solid phase extraction (SPE). This provides a first information concerning the chemical structure of the unknown compound.
In a first step SPE separates the raw extract into three different fractions of lipids: free fatty acids, polar lipids and neutral lipids. We identified the unknown olefinic signal within the neutral lipid fraction. Figure 3 shows $^1$H-NMR spectral sections of the olefinic domain of a lipophilic tissue extract before separation (blue) and $^1$H-NMR spectra of the three obtained fractions after SPE (free fatty acids in yellow, polar lipids in red and neutral lipids in green). In a second SPE step neutral lipids were separated into their subclasses using an eluent with increasing polarity. Cholesterylester, triglycerides, cholesterol as well as diglycerides and monoglycerides were eluted with increasing polarity. The unknown component appeared preferentially within the triglyceride fraction (80%), but so far we have no spectroscopic proof, that the unknown is a triglyceride. Surprisingly, the unknown olefinic signal shows also up within the cholesterylester fraction and the monoglycerides (~ 10% each). 2D spectra of the fraction with the highest content of the unknown compound confirmed the previous substructure determination.

## Conclusions

An unknown olefinic compound has been found in tissue extracts from the tumor core of astrocytic brain tumors but not in the tumor margins. This compound is a neutral lipid and no free fatty acid or polar lipid. Within the subclasses of neutral lipids the unknown compound resembles the triglycerides.

## References

[1] F.A. Howe et al., *NMR Biomed.* 2003; 16; 123-131
[2] R.J. Maxwell et al., *Magn. Reson. Med.* 1998; 39(6); 869-877
[3] M.A. Kaluzny et al., *Journal of Lipid Research* 1985; Vol 26; 135-140

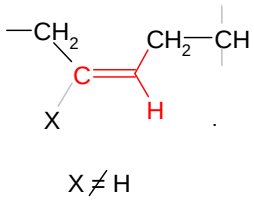


**Figure 2: assumed structure of the olefinic proton.**

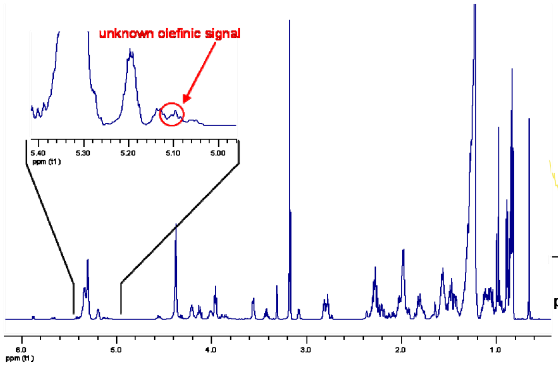


**Figure 1: $^1$H-NMR spectrum of a tumour core tissue extract with the highlighted signal of the unkown olefinic proton.**

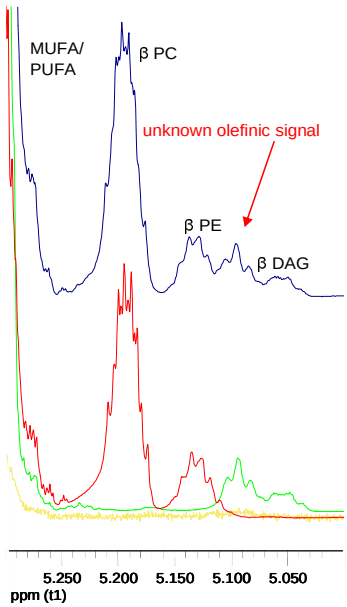


**Figure 3:$^1$H-NMR spectrum sections of the olefinic domain of lipophilic tissue extract before separation (blue) and after separation (fatty acid fraction yellow, polar lipid fraction red neutral lipid fraction green).**