MR profiles of cervial cancer biopsies correlate to apoptosis and cellular density

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Background

Elucidation of the biochemical pathways of apoptosis in cancers is believed to be important in the development of optimal treatment regimes. The potential of MR spectroscopy to detect apoptosis should be explored, as in vivo MRS might become a non-invasive method to monitor cancer therapy. Several studies have reported a change in lipids and cholines as a function of apoptosis [1 - 3]. We here report a high-resolution magic angle spinning (HR MAS) MR study on tumor samples from radiation and chemotherapy treated cervical cancer patients. MR characteristics of apoptosis in samples from cancer patients are highly relevant for a clinical setting, as the apoptotic frequency in such samples is representative for carcinomas.

Methods

Tumor samples (n=40) were collected from 20 patients with invasive cervical cancer (squamous cell carcinomas). For each patient, one tissue sample was collected before treatment started and a second sample was collected one week after treatment with radiation (10 Gy) and cisplatin (6 mg/m^2). Tissue specimens were stored at -80ºC. The samples were cut to fit a MAS rotor (50µL, mean sample weight 16.2 mg), and added PBS buffered deuterated saline with TSP. MAS spectra were recorded on a BRUKER AVANCE DRX600, at 5 kHz spinning speed and operating temperature of 4ºC. A single-pulse sequence (zgpr; BRUKER) was used. Areas were calculated using curve fitting (PeakFit, Jandel Scientific). The spectral regions 3.4 – 2.9 ppm and 1.8 – 0.5 ppm were fitted using a combined Lorentzian and Gaussian line shape (Voigt area). All calculated spectra correlated to their corresponding real spectrum with a correlation factor (r^2) of 0.95 or better. After MAS spectroscopy the samples were fixed in formalin, embedded in paraffin casts and cut into 5 µm thick sections. Fraction of carcinoma tissue and density of carcinoma cells were determined by point counting in HE stained sections. Frequency of apoptosis was determined in sections stained with Apotag in situ apoptosis detection kit (Oncor, Gaithersburg, MD). The apoptotic frequency was calculated relative to the number of carcinoma cells. The total apoptotic frequency was determined by including fraction of carcinoma cells in the calculation.

Results

The MR spectra showed broad resonances from lipids and macromolecules, as well as sharp peaks from smaller compounds, like lactate, amino acids and cholines. Apoptotic frequency ranged from 0 (ten samples) to 12 (mean value 1.4), whereas fatty acid CH_2/CH_3 ratios ranged from 1 to 4.6 (mean value 2). Lipid-CH_2/CH_3 ratio showed weak correlations (Pearson) to cellular density (r^2: -0.33, p: 0.032) and total apoptotic frequency (r^2: 0.30, p: 0.045). Different ratios of cholines to lipid-CH_3 showed significant correlations to cellular density. The strongest correlation was found for total cellular density to the ratio of total cholines (sum of glycerophosphocholine, phosphocholine and choline) to lipid-CH_3 (r^2: 0.50, p: 0.001).

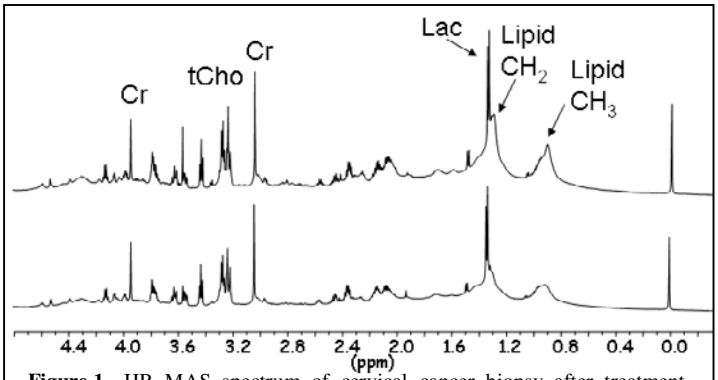

Figure 1 HR MAS spectrum of cervical cancer biopsy after treatment (upper spectrum) and before treatment (lower spectrum) from the same patient. Abbreviations: Cr; creatine, tCho; cholines and lac; lactate. The sample excised after treatment had higher apoptotic frequency and lower cellular density than the sample collected before the treatment started. The lower spectrum showed a smaller Lipid-CH_2/CH_3 ratio (1.49) than the upper spectrum (2.04), and a higher tCho/Lipid-CH_3 (0.20 compared to 0.16). These findings are consistent with the results from our correlation analysis.

Discussion

Our findings show a weaker correlation between the content of cholines and lipids to apoptotic frequency in cervical cancer tissue than previous reports on cell cultures and animal models. However, the apoptotic densities in the cervical specimens studied here were lower (Blankenberg[1] studied cell cultures containing 8 to 60% apoptotic cells). As multiple other factors, like cell density and tumor fraction, might influence the spectra, thorough analysis of the data must be done in order to understand the biochemical aspects.

Conclusion

There is a correlation between the content of cholines and lipids observed by MRS and apoptosis and cellular density in cervical cancer tissue. We believe HR MAS MRS of tissue biopsies will provide valuable information when exploring the potential of in vivo MRS as a non-invasive method to monitor apoptosis.

References

1. Blankenberg FG et al. *Blood*. 1997; **89**: 3778-3786. 2. Valonen PK et al. *NMR Biomed*. 2005; **18**: 252-259. 3. Hakumaki JM et al. *Nat Med*. 1999; **5**: 1323-1327.