Predicting long term survival for breast cancer patients by HR MAS metabolic profiling during neoadjuvant chemotherapy

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Introduction

Metabolic profiles obtained from MR spectra of breast cancer tissues have proven to correlate to important clinical parameters, such as tumor grade, lymphatic spread and hormone status[1,2]. In addition, decreases in total choline (tCho) signals detected by in vivo MRS have been found during neoadjuvant chemotherapy (NAC) in breast cancer patients[3]. The purpose of this study was to evaluate whether MR metabolic profiles can assist the prediction of long time survival in locally advanced breast cancer patients.

Experimental

Tissue samples from 19 patients diagnosed with locally advanced breast cancer were included in this study. The patients were treated weekly with doxorubicin (14mg/m^2) for 16 weeks. Pairs of tissue samples (16.6 ± 3.4 mg) obtained pre (n=19) and post treatment (n=19) were analyzed by high resolution magic angle spinning (HR MAS) MRS on a Bruker AVANCE DRX600 spectrometer (spin rate 5 kHz, 4 °C). Spin-echo spectra (cpmgpr; Bruker) and pulse-acquired spectra including the ERETIC quantification reference (ereticpr.drx; Bruker) were recorded for all samples as previously described[1,4]. Preprocessing methods including baseline correction, mean-normalization, mean centering, and peak alignment were performed prior to data analysis. From the spin-echo spectra, metabolite regions of interest (1.43-1.53 ppm, 2.90-3.65 ppm and 3.75-4.7 ppm) were related to clinical outcome by partial least squares regression discriminant analysis (PLSDA). PLSDA was performed with leave one out cross-validation. The metabolite concentrations of GPC, PCho, Cho, Gly and Tau were calculated from peak areas obtained by curve fitting (PeakFit, Seasolve; USA) in the pulse-acquired spectra. All tissue samples were confirmed to contain tumor cells by histopathology analysis.

Results and Discussion

The PLSDA score plots of HR MAS spectra obtained pre and post treatment showed a clear discrimination between patients with long time survival ≥5 years (survivors, n=13), and patients who died of cancer recurrence within 5 years (non-survivors, n=6) (Figure 1A). The best classification accuracy was obtained using the post treatment data (Table I). Spectral data of survivors were tighter clustered after treatment, indicating that their metabolic profiles had been changed towards a more homogeneous distribution in response to doxorubicine. In addition, an overall decrease in mean tissue concentrations of metabolites was also observed (Table II). High lactate and glycine signals suggest potentially higher grade of malignancy and more aggressive tumors in the non–survivors group after treatment (Figure 1B). High glycine signals in MR spectra have been associated with high grade brain tumors[5] and tumor aggressiveness in breast cancer[1]. High lactate concentrations have been associated with increased risk of metastasis and poorer outcome in several cancer types[6,7]. Survivors had a higher tCho before treatment compared to non-survivors. However, large variations were observed and tissue concentrations of tCho were not significantly different between the two groups. Significant decrease in GPC and Cho concentrations were observed in survivors post treatment, suggesting these metabolites as potential biomarkers of effective long term response to NAC (Table II).

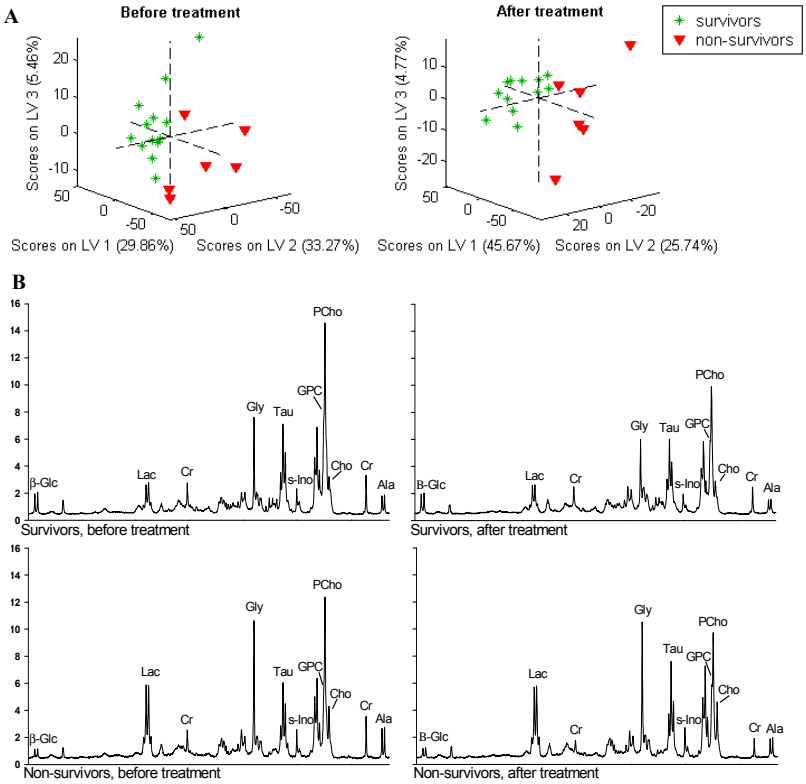

Figure 1. (A) PLSDA score plots, pre (left) and post (right) treatment. (B) Average metabolic profiles of survivors (n= 13, top) and non survivors (n= 6, bottom), pre (left) and post (right) treatment. *Abbreviation used: β-glucose (β-Glc), lactate (Lac), creatine (Cr), glycine (Gly), taurine (Tau), scyllo-inositol (s-Ino), glycerophosphocholine (GPC), phosphocholine (PCho), choline (Cho) and alanine (Ala).*

Table I. PLSDA classification results

Before treatment				After treatment			
Total variance (X/Y)	Sens. (CV)	Spec. (CV)	Class. Err. (CV)	Total variance (X/Y)	Sens. (CV)	Spec. (CV)	Class. Err. (CV)
68.6%/73.3%	69.2%	66.7%	32.1%	76.2%/80.3%	84.6%	83.3%	16.0%

Table II. Quantification results (mean±SD) µmol/g

	≥ 5 years survival (n=13)			< 5 years survival (n=6)		
Metabolites (ppm)	Before treatment	After treatment	Paired T-test	Before treatment	After treatment	Paired T-test
GPC (3.24)	**1.26±1.48**	**0.79±1.09**	**0.025**	0.81±1.71	0.51±0.52	0.446
PCho (3.23)	2.36±5.44	1.60±4.06	0.356	0.83±1.48	0.68±0.65	0.627
Cho (3.21)	**0.50±0.98**	**0.33±0.64**	**0.036**	0.22±0.15	0.23±0.06	0.897
tCho	4.13±7.52	2.71±5.65	0.143	1.86±2.54	1.42±1.01	0.439
Gly (3.55)	0.48±0.81	0.29±0.37	0.082	0.34±0.58	0.59±1.22	0.530
Tau (3.42)	1.39±3.05	0.99±2.02	0.136	0.46±0.55	0.92±1.53	0.350

Conclusion

Different metabolic profiles have been observed for breast cancer survivors and non-survivors, which could be related to tumor malignancy and aggressiveness. High Cho and GPC before treatment were associated with better outcome. We suggest that breast cancer metabolic profiles have the potential to assist the prediction of prognosis in locally advanced breast cancer patients.

References (1) Sitter et al. NMR Biomed 2006;19(1):30-40, **(2)** Bathen et al, Breast Cancer Res Treat 2007;104(2):181-9, **(3)** Meisamy et al. Radiology 2004;233(2):424-431, **(4)** Sitter et al, NMR Biomed 2009 (in press), **(5)** Davies et al, NMR Biomed 2009, (Epub ahead of print) **(6)** Brizel et al, Int J Radiat Oncol Biol Phys 2001;51:349-53, **(7)** Walenta et al, Cancer Res 2000;60:916-21.