Choline metabolites as biomarkers for predicting response to neoadjuvant chemotherapy in local advanced breast cancer patients

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Introduction

Early prediction of response to chemotherapy is important to avoid ineffective therapy and unnecessary side effects. Anthracyclines, like doxorubicin, are among the most effective chemotherapeutics in breast cancer. The presence and change in the composite choline signal detected in MR spectra has been suggested as a biomarker for breast cancer diagnosis and treatment monitoring (1,2,3). Choline (Cho) affect the cell signaling and lipid metabolism, while the choline metabolites i.e. glycerophosphocholine (GPC) and phosphocholine (PC) assure the structural integrity and signaling functions of cell membranes (2). The purpose of this study was to evaluate quantitative changes in the level and composition of the choline-containing metabolites (tCho) prior to and after treatment with doxorubicin monotherapy in 30 patients with breast cancer.

Experimental

In this study, 30 patients with locally advanced breast cancer (TNM-stages T_1-T_3) were treated weekly with doxorubicin (14mg/m^2) for 16 weeks. Tumor diameters were measured with calipers and the clinical response was classified according to UICC criteria (4). Patients with a >50% reduction in tumor volume were considered responders (n=11), while patients with a <50% reduction or <25% increase in tumor volume were designated non-responders (n=19). Tissue specimens (16,9 ± 5,1 mg) attained from open surgical biopsy before starting on chemotherapy (n=30) and during main surgery (n=30) were analyzed by high resolution magic angle spinning (HR MAS) MRS on a Bruker AVANCE DRX600 spectrometer (spin rate 5 kHz, 4 °C). A pulse-acquired experiment including the ERETIC sequence (ereticpr.drx; Bruker) as a quantification reference was performed for all samples. Additionally, spin-echo spectra were recorded as previously described (5). The spin-echo spectra (using the region 1.4-1.6 ppm and 2.9-4.7 ppm) were related to clinical response by partial least squares regression (PLS). PLS was performed with full cross-validation and mean-centered data. The GPC, PC and Cho concentrations were calculated from peak areas obtained by curve fitting (PeakFit) in the pulse-acquired spectra.

Results and Discussion

In the PLS analysis, the predicted versus clinical response were significantly correlated both for calibration (r=0.59, p<0.01) and validation (r=0.38, p<0.05) (Figure 1). The PLS score plot of PC1 and PC2 shows clustering of spectra from both the responder and non-responder groups, defining 4 sub-groups with different choline profiles (Figure 1). Calculated average spectra (only choline region shown) of the responder group with choline profiles 1 and 2, and the non-responder group with choline profiles 3 and 4 shows dissimilar compositions of choline compounds. Responders with profile 1 had significant decreased concentration of GPC and PC (p<0.01) (Table 1), consistent with previous in vivo MR studies of breast cancer patients where decreased level of total choline reflects inhibition of cellular proliferation (3). Conversely, responders with profile 2 had a higher concentration of GPC compared to PC before treatment (Table 1), and no decrease in PC after treatment. Microenvironmental condition of the tumor, such as acidosis and hypoxia, can affect the choline metabolism (2). Non-responders with profile 3 had lower concentration (not significant) for all the choline metabolites before chemotherapy compared to after; this may be related to the resistance to doxorubicin and increased proliferation. The average spectra of profile 4 shows a decrease level of tCho after treatment, however the quantification of the different metabolites showed that the reduction of GPC was the most important (p=0.09).

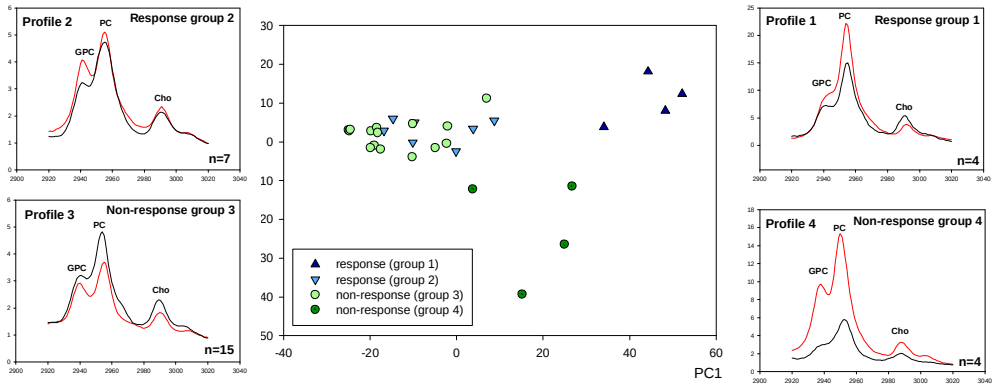

Figure 1. Results from PLS and average spectra. Score plot of PC1 versus PC2 from PLS of spectra (n=30) obtained before chemotherapy. Average spectra of responders (profile 1 and 2) and non-responders (profile 3 and 4), before (red) and after (black) chemotherapy.

Table 1 Average tissue metabolite concentration (µmol/g) (± standard deviation) calculated in samples excised before and after chemotherapy for 16 of the patients. Four patients were chosen by random for the four subgroups defined by PLS.

	GPC			PC			Cho		
	Before	After	T-test	Before	After	T-test	Before	After	T-test
Group 1 (n=4)	1.75 (±0.35)	0.60 (±0.50)	**p= 0.01**	4.36 (±1.46)	1.23 (±1.07)	**p= 0.01**	0.55 (±0.16)	0.34 (±0.17)	p= 0.12
Group 2 (n=4)	1.92 (±0.94)	0.79 (±0.44)	***p= 0.07***	1.14 (±0.15)	1.91 (±2.17)	p= 0.50	0.54 (±0.26)	0.49 (±0.37)	p= 0.85
Group 3 (n=4)	0.61 (±0.73)	1.02 (±0.84)	p= 0.49	0.49 (±0.34)	0.90 (±0.72)	p= 0.34	0.36 (±0.23)	0.22 (±0.12)	p= 0.34
Group 4 (n=4)	4.25 (±2.05)	1.45 (±1.93)	***p= 0.09***	8.42 (±7.53)	5.26 (±7.09)	p= 0.56	1.75 (±1.62)	0.84 (±1.14)	p= 0.40

Conclusion

Different choline profiles and concentration were identified in responders compared to non-responders treated with doxorubicin. The differences were most significant for GPC, but also severe changes in PC and Cho were seen. Although further studies are needed to validate these data, our study suggests that quantitative changes in different choline profiles may be related to breast cancer treatment response.

References

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