Tissue metabolic concentrations investigated with respect to prognosis for breast cancer patients

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Background

In the search for improved treatment and care for breast cancer patients, understanding breast cancer biology is important. Cancer pathways have been explored using cancer cell lines and animal models (1-3). However, how these model systems reflect human breast cancers is not fully understood. For molecular mechanisms studied in vitro to be translated to clinical use, the metabolic information should be quantitative and relate to clinically relevant parameters. The purpose of this study was to evaluate the correlation of tissue metabolite concentration to prognostic factors and patient survival data in breast cancer.

Experimental

Breast cancer samples were analyzed from patients with good prognosis (n=7, ER and PgR +, negative lymph nodes and tumors < 2 cm), poor prognosis (n=7, ER or PgR -, positive lymph nodes and tumors > 2 cm) and from diseased patients (n=3, survival < 5 years). 3,3 mg of deuterated PBS with 10 mM TSP was added to leak-proof disposable Kel-F inserts (Bruker) before adding the tissue sample (16,9 ± 5,1 mg). Three different solutions of creatine (10, 5, and 1 mM) in PBS with TSP (10 mM) were prepared for quantification and calibration of the ERETIC signal. HR MAS experiments were performed on a Bruker AVANCE DRX600 spectrometer (spin rate 5 kHz, 4 °C). A pulse-acquired experiment including the ERETIC sequence (ereticpr.drx; Bruker) was performed for all solutions and samples (SW 16.7 ppm, NS 64, TD 64K, AQ 3.28 s). The ERETIC signal was obtained using a 40 dB attenuator and a pulse level of 35 dB, positioned at -1.0 ppm. Peak areas of creatine, TSP and ERETIC signals in spectra of solutions, and glycine, glycerophosphocholine (GPC), phosphocholine (PC) and choline signals in spectra from tissue samples were calculated by curve fitting (PeakFit, SeaSolve Software Inc). The ERETIC signal was calculated from the CH_3-signal in spectra (n=3) of the 10 mM creatine. This value was calibrated using three spectra from each of the three different solutions of creatine. Metabolite concentrations and peak area ratios were compared to patient outcome and prognostic factors by ANOVA using Bonferoni post hoc test (SPSS).

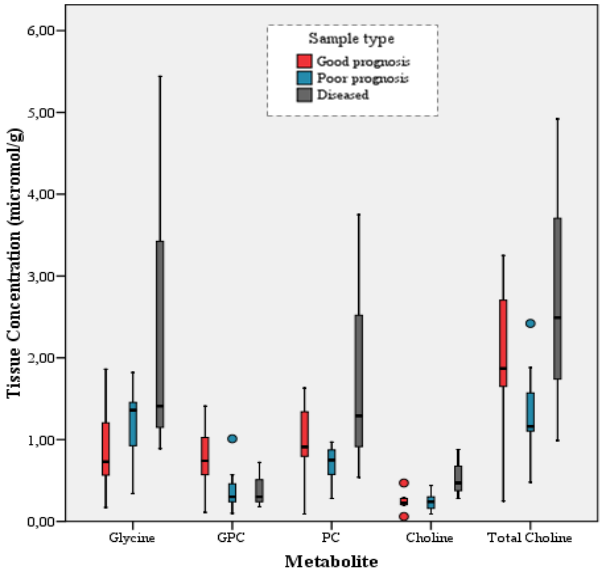

Figure 1 Box plot of metabolite concentrations in breast cancer tissue. Outliers are presented as circles.

Results

Calibration of the ERETIC signal showed that creatine concentrations were calculated by an accuracy of at least 5.2% using the ERETIC signal. Tissue metabolite concentrations and peak area ratios are shown in Table 1, and tissue metabolite concentrations are presented graphically in Figure 1. We found the trends that tissue from patients with poor prognosis had higher GPC compared to the good prognosis group and lower PC compared to the diseased group. Significant differences between groups of samples were also found for ratios of GPC/Gly and PC/GPC. Samples from diseased patients showed significantly higher concentrations of choline than samples from patients with good and poor prognosis.

Discussion and conclusion

Tissue metabolic concentrations and metabolite peak area ratios correlate to patient outcome and prognostic factors, suggesting differences of metabolic concentrations, despite variance in tissue composition (4). Increased GPC has not been associated with tumor aggressiveness, but can be related to tumor microenvironment (3). High choline concentration in samples of diseased patients is interesting and should be further investigated on a larger number of samples. The sample is intact after HR MAS analysis and can be further investigated by histopathology. Using this combined analytic strategy on a larger sample material would enable the evaluation of the influence of tissue composition and tumor micro environment.

Table 1 Average metabolite concentrations (µmol per gram) ± SD and average ratios of metabolite areas ± SD in breast cancer tissue from patients with good prognosis, poor prognosis and diseased patients. Significantly different ($p<0,05$) concentrations are labeled with an asterix.

	Glycine	GPC	PC	Choline	Total Choline	GPC/Gly	PC/Gly	Cho/Gly	PC/GPC
Good prognosis (n=7)	0,90±0,57	0,78±0,43	0,97±0,51	0,25±0,12	2,01±1,00	4,14±1,66*	5,10±2,02	1,42±0,56	1,26±0,36
Poor prognosis (n=7)	1,18±0,50	0,40±0,30	0,70±0,26	0,24±0,12	1,34±0,63	1,58±0,92	3,41±2,91	1,1±0,85	2,23±1,04
Diseased (n=3)	2,58±2,49	0,40±0,29	1,86±1,68	0,54±0,30*	2,80±1,98	1,15±1,05	3,31±0,73	1,22±0,43	5,93±5,93*

References

(1) Aboagye EO *et al.* Cancer Res **59** (1999) **(2)** Glunde K *et al.* Expert Rev Mol Diagn **6** (2006) **(3)** Eliyahu G *et al.* Proc Intl Soc Mag Reson Med **15** (2007) **(4)** Sitter B *et al.* NMR Biomed **19** (2006)