Combined HR MAS MR spectroscopy and gene expression of breast cancer tissue

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Background

Genetic portraits of breast cancer have led to the identification of five different groups of patients which show distinct survival curves (Perou et al. 2000). The biochemical activity in cancer tissue is also altered and HR MAS spectral profiles of breast cancer tissue correlate to clinical findings (Bathen et al. 2006). Since tissue samples are intact after HR MAS analysis, the sample can be further analyzed with micro array technology. The purpose of this study was to inspect if the HR MAS derived biochemical pattern reflect the genetic expression in the same breast cancer specimens.

Experimental

Tissue samples were collected from breast cancer patients (n=47) and stored in liquid nitrogen until analysis. Two samples from each patient were analyzed by HR MAS. Samples (av. weight 20.5 mg) were cut to fit a 4 mm zirconium rotor PBS-TSP buffer. HR MAS experiments were performed on a Bruker AVANCE DRX600 spectrometer (5 kHz spin, 4° C) and spin echo experiments were performed using TE=285 ms. The ratios PC/GPC, PC/choline and GPC/choline were determined by peak intensity measures. After MR analysis, half the samples were fixed in 10% formalin and embedded in paraffin to be analyzed by histopathology. The relative areas of normal and neoplastic epithelial elements, necrotic tissue, fat and fibrous connective tissues were scored by a pathologist. At least 5% tumor tissue was used as inclusion criteria for micro arrays

Total RNA was extracted from tumor tissue (n=41) following the protocol for the RNeasy Mini Kit (Qiagen), quantified by spectrophotometry (Nanodrop) and the integrity of total RNA was controlled by electrophoreses (BioAnalyzer 2100, Agilent). Good quality RNA was obtained from 37 samples, which was amplified and labeled using Agilent Low RNA Input Fluorescent Linear Amplification Kit (Agilent Technologies). Total tumor RNA was labeled by Cy5 and reference RNA (Universal Human Reference total RNA, Stratagene) was labeled by Cy3. Labeled cRNA of good quality was obtained from 32 samples, which was hybridized to Human Whole Genome Oligo Microarrays (Agilent, 60-mer oligo microarray processing protocol) and scanned on an Agilent Microarray Scanner G2505B. Three of the arrays contained more than 10% outliers (Feature Extraction v. 8.5, Agilent Technologies) and was excluded from further analysis, leading to a total of 29 arrays. Array data were analyzed by GeneSpring GX 7.3.1 (Agilent Technologies). Choline associated genes (82) were identified and normalized intensities were compared to ratios of choline-compounds from MAS spectra by Pearson correlation analysis (SPSS).

Results and Discussion

Significant correlations ($p < 0.05$) were found for choline metabolite ratios from MAS spectra to eight of the choline associated genes (Table 1). One of these genes (NM_001263) was upregulated (intensity > 2) in 24 of the 29 samples, whereas NM_000903 was downregulated (intensity <0.7) in 28 of the 29 samples. In a study combining MR spectroscopy and genetic expressions on cell cultures (Eliyahu et al. 2007) the specific choline transporter CHT1 (NM_021815) was reported upregulated in breast cancer cells in comparison with that in the normal mammary epithelial cells. We found no altered genetic expression for this gene (average normalized signal intensity: 1.05). MR spectra and micro arrays of breast cancer samples contain vast amounts of information, and further data analysis is needed in order to inspect correlations between biochemical information from MAS spectra and genetic expression from micro arrays.

Table 1 Mean normalized intensity for 29 breast cancer samples and Pearson correlation factor (p-value in brackets) to choline-ratios from MAS spectra for eight genes.

GenBank Accession #	Description	Intensity (mean)	PC/GPC	PC/Cho	GPC/Cho
NM_000750	Cholinergic receptor, nicotinic, beta polypeptide 4 (CHRNB4)	0.98		0.42 (0.023)	
NM_002662	Phospholipase D1, phosphatidylcholine-specific (PLD1)	1.26			-0.50 (0.005)
NM_000903	NAD(P)H dehydrogenase, quinone 1 (NQO1), transcript variant 1	0.33	0.70 (<0.001)	0.38 (0.041)	-0.46 (0.011)
NM_001263	CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 1 (CDS1)	8.04		0.37 (0.048)	
NM_020549	Choline acetyltransferase (CHAT), transcript variant M	1.36		0.62 (<0.001)	0.37 (0.048)
NM_000041	Apolipoprotein E (APOE)	1.71		0.41 (0.027)	
NM_000665	Acetylcholinesterase (YT blood group) (ACHE), transcript variant E4-E6	1.36			-0.44 (0.016)
NM_000748	Cholinergic receptor, nicotinic, beta polypeptide 2 (neuronal) (CHRNB2)	1.34		-0.37 (0.048)	

Conclusion

Breast cancer tissue intensity ratios of choline compounds correlate to the genetic expression of selected choline associated genes.

References

Bathen et al., *Breast Cancer Res Treat* (2006), **Eliyahu et al.,** *Int. J. Cancer* (2007), **Perou et al.,** *Nature* (2000)