Choline metabolic composition correlates to basal-like and luminal A genetic subtypes in orthotopic breast cancer xenografts

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Introduction

Molecular sub-classification of breast cancer based on gene expression pattern represent clinically distinct patient groups.[1] Two new breast cancer xenograft model systems, reflecting the basal-like (poor prognosis) and luminal A (better prognosis) subgroups, have recently been established corresponding to this classification.[2] MR spectroscopy has demonstrated altered choline metabolism in malignant breast cancer lesions.[3-5] It has also been shown that elevation of the choline metabolites phosphocholine (PC) and glycerophosphocholine (GPC) are associated with malignant transformation of breast cancer cell lines.[3-5] The purpose of this work was to correlate metabolic patterns to gene expression profiles in two animal tumor models of human origin using HR-MAS MRS with the ERETIC method for metabolic quantification.

Methods

Luminal A (MAS 98.06) and basal-like (MAS 98.12) breast tumor cells were collected from human breast cancer, and orthotopic xenograft models in female BalbC nu/nu mice established[2]. Ten tumors from each model were harvested at a diameter of 13-15 mm (90 and 48 days, respectively). Biopsies (15 ± 3 mg) were analysed using HR-MAS MRS performed on a Bruker AVANCE DRX 600 spectrometer (Bruker BioSpin, Karlsruhe, Germany). Single-pulse spectra were obtained according to previously described procedures[6] including ERETIC for quantitation.[7] Peak areas were calculated using peak-fitting software (PeakFit v 4.12 by Jandel Scientific, Chicago, USA) and selected metabolites were quantified. The HR MAS results were compared to gene expression data and histopathology.

Results

The gene expression results showed several clusters of genes differently expressed by xenografts and primary tumors. However, both xenograft models maintained the intrinsic "molecular subtype" classification[2]. Histopathological analyses showed that the two xenograft models retained the morphological characteristics of the original human breast tumors, which both were classified as invasive ductal carcinoma grade III. The luminal A xenografts showed strong positive staining for the ER and PgR hormone receptors while the basal-like model was ER and PgR receptor negative. The basal-like xenografts had a higher fraction of necrosis.The HR MAS results revealed several significant differences in the metabolic profiles of the two xenografts. The concentrations of selected metabolites are presented in Table 1, and representative spectra in Figure 1.

Figure 1 Example of HR MAS choline metabolite pattern in MAS98.12 (left) and MAS98.06 (right) xenografts

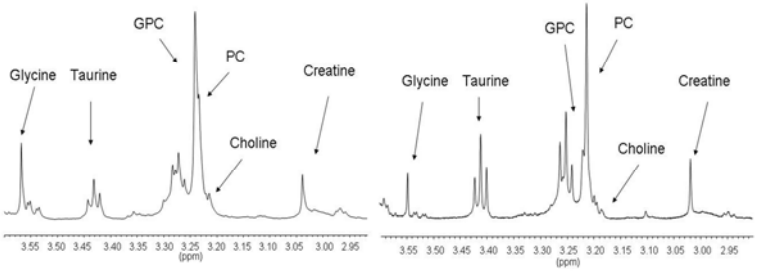

Table 1 Metabolite concentrations in tumor samples
(mean ± SD, µmol/g, * p<0.02, ** p<0.001)

	MAS98.12 (n=10)	MAS98.06 (n=9)
Creatine	4.1 ± 1.4	3.4 ± 1.7
Choline	1.2 ± 0.7	0.9 ± 0.6
Phosphocholine *	4.8 ± 1.7	9.1 ± 4.4
Glycerophosphocholine **	9.8 ± 2.5	2.7 ± 1.7
Taurine	14.7 ± 4.1	19.1 ± 9.1
Glycine *	8.2 ± 2.9	4.0 ± 1.8

The metabolite concentrations measured in the two tumor models were compared using a two-sided unpaired t-test. There was a marked difference in both GPC and PC concentrations. In the luminal A group, all tumors had a low GPC/PC ratio compared to the basal-like group (Mean 0.3±0.1 vs 2.2±0.9, respectively. p<0.00001). Significant difference in glycine concentration was also found.

Discussion

There were significant differences in metabolite concentrations between the two breast tumor xenograft models. The choline metabolite pattern shifted towards higher GPC and lower PC in the fastest growing basal-like xenograft model. These findings might reflect an intrinsic difference in choline metabolism between the basal-like and the luminal A breast cancer subgroups, or be a result of differences in tumor microenvironment. It has previously been shown that the GPC/PC ratio can be modulated *in vitro* by environmental factors such as acidosis or hypoxia[8]. The data from our study suggest that a high GPC/PC ratio is associated with fast-growing, aggressive tumors and the xenografts will be further investigated by immunohistochemistry with respect to microenvironmental factors. In conclusion, the genetic expression profiles of the established tumor xenograft models represent the basal-like and luminal A molecular subgroups of human breast cancer, and the two models had significant differences in choline metabolism pattern. Further studies of these xenograft models are warranted in order to explore the prognostic value of the choline metabolic composition in different molecular subgroups of breast cancer.

References

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