Interplay of choline metabolites and genes in patient-derived breast cancer xenografts

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Target audience: Basic scientists involved in identification of metabolic biomarkers or new drug targets in cancer.

Purpose: Abnormal choline metabolism is a renowned feature of breast cancer, but the underlying mechanisms are not fully understood. The purpose of this study was to explore the metabolomic and transcriptomic characteristics of a large panel of patient-derived breast cancer xenografts, with special attention on choline metabolism and to evaluate the clinical relevance of xenograft models for metabolomic studies.

Methods: Tumor tissue specimens were obtained from patient-derived xenograft models (N=34).[1] and used for both high-resolution magic angle spinning (HR MAS) MR spectroscopic analysis and gene expression microarrays. HR MAS MRS was performed on a Bruker Avance DRX600 spectrometer using water presaturation sequence (Bruker: ^{1}H zgpr). Experiments were performed at 5ºC with a spin rate of 5kHz. The correlation between levels of choline (Cho), phosphocholine (PCho) and glycerophosphocholine (GPC) with expression of genes encoding proteins in the choline metabolism was investigated using Pearson's correlation analysis. Metabolic and gene expression data were also retrieved from a human breast cancer tissue biobank to evaluate the relevance of xenograft models.

Results: Most of the xenograft models were classified as basal-like (N=19) or luminal B (N=7) breast cancer, with significantly different choline metabolic and gene expression profiles. The luminal B xenografts were characterized with a high PCho/GPC ratio (2.5±0.9) while the basal-like xenografts were characterized with highly variable PCho/GPC ratio (1.9±1.5) (Figure 1). The same metabolic pattern was observed in ER positive and ER negative human tissue samples (Figure 1). Figure 2 shows Principle component analysis on the MR spectra from xenografts. Most of the luminal B samples showed higher levels of PCho, creatine, taurine, glycine and lactate, and lower levels of GPC and Cho compared to basal-like samples. Further, Cho, PCho and GPC levels were correlated to expression of several genes involved in choline metabolism, including choline kinase alpha (*CHKA)* and glycerophosphodiester phosphodiesterase domain containing 5 (*GDPD5)*. A strong correlation was observed when expression of genes in the choline metabolism pathway was compared in xenografts and human tissue samples as shown in Figure 3.

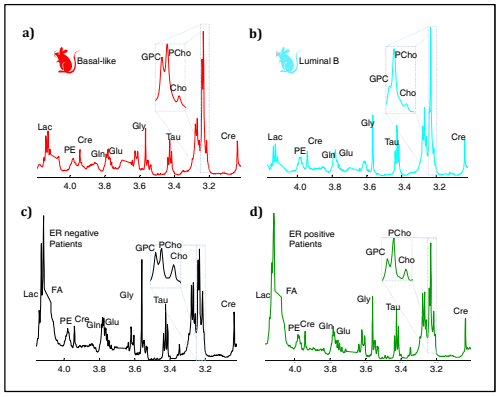

Figure 1: Mean MR spectra; a) basal-like xenograft samples, b) luminal- B xenograft samples, c) ER negative breast cancer patient samples and d) ER positive breast cancer patient samples.

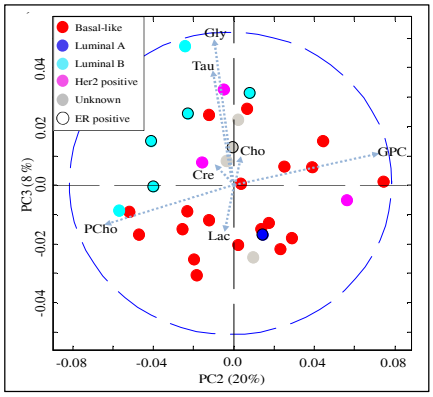

Figure 2: Combined PCA score plot and loading profiles (Bi- plot) from HR-MAS MR spectra of xenografts (N=33).

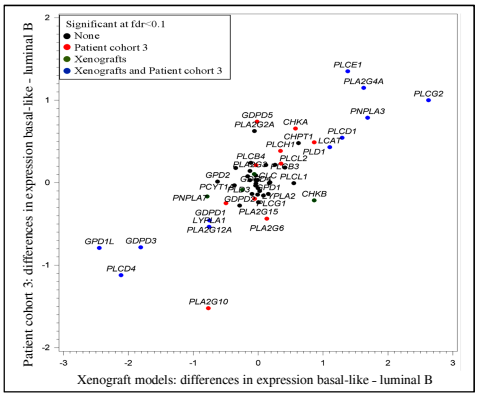

Figure 3: Comparison of choline gene expressions between basal-like and luminal B xenografts and human tissue samples (Cohort 3).

Discussion: The higher PCho/GPC ratio in the luminal B compared to basal-like breast cancer xenograft models and human tissue samples is not consistent with earlier *in vitro* studies.[2] It is likely that microenvironmental factors may play a role in the *in vivo* regulation of choline metabolism.[3] In addition, the differences in Cho, PCho and GPC levels between luminal B and basal-like xenograft samples, suggest that regulation of choline metabolism may vary between different breast cancer subtypes. The concordance between the metabolic and gene expression profiles from xenografts and human tissue samples indicates that these xenografts are representative models of human breast cancer and represent relevant models to study tumor metabolism *in vivo*.

Conclusion: Metabolic and gene expression analyses indicate that the patient-derived xenografts are representative of human breast cancer, and may be valuable for further exploration of subtype-specific metabolic and transcriptomic traits. In addition, the models are relevant for studies of targeted anticancer drugs and molecular properties associated with sensitivity and resistance to chemotherapy.

References: 1] Marangoni E, Vincent-Salomon A, Auger N, et al. A new model of patient tumor-derived breast cancer xenografts for preclinical assays. *Clin Cancer Res*. 2007; 13(13): 3989-98. 2] Aboagye EO, Bhujwalla ZM. Malignant transformation alters membrane choline phospholipid metabolism of human mammary epithelial cells. *Cancer Res*. 1999; 59(1): 80-84. 3] Mori N, Glunde K, Takagi T, Bhujwalla ZM. The tumor microenvironment alters choline phospholipid metabolism detected by comparing cancer cells with tumors: [abstract]. In: *Proc Intl Soc Mag Reson Med*. 2008; 16:2008.