Metabolic subgrouping of breast cancer using HR MAS MRS and hierarchical cluster analysis; correlation with molecular subtypes

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Target audience: Basic scientists investigating metabolomic biomarkers for cancer prognosis and treatment or breast cancer subtypes

Purpose: The heterogeneous biology of various breast tumors has led to the need for detection of clinically relevant subgroups. In the past decades, the use of different high-throughput genome-wide profiling techniques has identified several novel molecular genetic events, and subsequently their biological and clinical impact has been validated[1,2]. Here we show the resulting metabolic subgroups using hierarchical clustering of the MR metabolic profiles from tumor biopsies from breast cancer patients. These were combined with data from gene expression and reverse phased protein arrays (RPPA) from the same patients to search for any relationship between metabolic profiles and molecular subtypes and relate these to clinical information. This novel approach bridging MR metabolic subgroups to gene expression and protein profiles may improve our knowledge about various classes of breast cancer that may contribute to personalized treatment.

Methods: The metabolic profiles of a large cohort of primary tumors from breast cancer patients (N=280 tissue samples) were determined using ex vivo HR MAS MRS. Principal component analysis (PCA) was used to extract the metabolically important variance structure before hierarchical cluster analysis using Ward's method. RPPA and unsupervised hierarchical cluster analysis was used to carry out a protein-based subtype classification of the tumor samples. In addition, the samples were classified into expression subtypes based on prediction analysis of microarray using a 50-gene classifier, the PAM50 method[3]. The distribution of metabolic cluster classes within the expression and the RPPA subtypes was evaluated to establish metabolic characteristics that could be associated to each breast cancer subtype.

Results: Four clusters based on metabolic differences were evaluated and correlated with expression and RPPA breast cancer subtypes. Comparison of the mean spectra for each of these clusters (Figure 1) revealed the main differences to be the levels of glucose, ascorbate, alanine, creatine, lactate, taurine, choline, PCho, GPC, glycine and lipids (Table 1). Metabolic cluster 3 was composed of around half of the samples. From the total cohort, 245 and 221 samples were classified into breast cancer subtypes using the RPPA and PAM50, methods, respectively. The distribution of metabolic cluster classes within RPPA subtypes (Figure 2) revealed that 74% of Reactive I samples were grouped into cluster 3. As for expression subtypes, only in Luminal B were the majority of samples not grouped into this cluster (Figure 2).

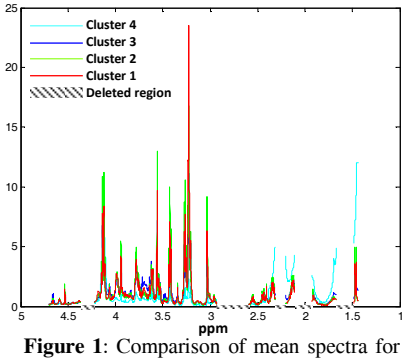

Figure 1: Comparison of mean spectra for the four metabolic clusters. Five regions have been omitted as shown due to high lipid signals.

Table 1: Main differences in metabolite levels between mean spectra from the four clusters. Arrow pointing up represents cluster where the metabolite level is higher when compared to the mean quantity from all samples. Arrow pointing down indicates lower level.

Metabolite	Cluster class			
	1 (n=64)	2 (n=62)	3 (n=138)	4 (n=16)
Al		↑	↓	
Cr		↑	↓	
Cho		↑	↓	
PCh, GPC	↑		↓	
Tau		↑	↓	
Gly		↑	↓	
Lac		↑	↓	
Asc		↑	↓	
Glc	↓	↓	↑	
Lipid content				↑

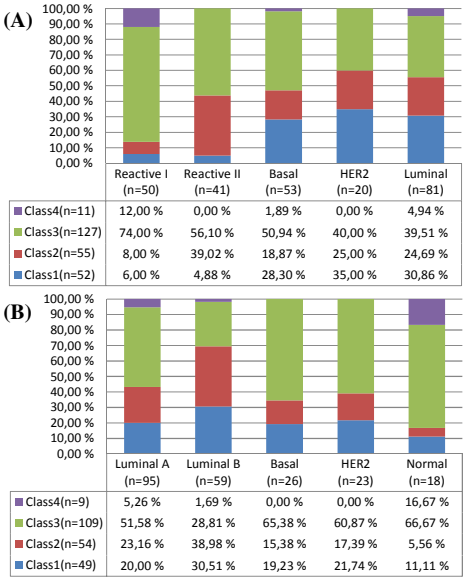

Figure 2: Relation between metabolic cluster classes and breast cancer subtypes classified (**A**) based on RPPA subtyping and (**B**) using the PAM50 centroid-based prediction method.

Discussion: Here we report four metabolic subgroups of breast carcinomas based on a large cohort of samples. These subgroups show differences in metabolites that previously have been found important in breast cancer. It has been shown that breast tumors have altered concentration of the metabolites such as choline, phosphocholine (PCho), glycerophosphocholine (GPC), lactate and glycine when compared to normal tissue[4]. When associating metabolic characteristics with expression subtypes, GPC/PCho ratio has been found to be higher in basal like versus luminal like breast cancers[5]. In this study, the most prominent congruence between breast cancer subtypes and metabolic subgroups was the enrichment of Reactive I samples in cluster 3, which was characterized by having higher glucose content (Table 1). However, this metabolic characteristic was found in a significant number of samples with different subtype; thus, there does not seem to be an association between metabolic profiles and breast cancer subtypes. This might make it possible for molecular subtypes to be further divided into subclasses based on metabolic differences. Metabolic data has previously been combined with transcriptomics data from the same samples to subclassify the Luminal A subtype into three groups[6]. The metabolic subgroups obtained in this study will now be further characterized by investigating the gene expression patterns from the same tumors. Furthermore, clinical data is available for the whole cohort, and this will be used to detect frequencies of traditional prognostic and predictive factors within the clusters.

Conclusion: Bridging information from several molecular levels in the same tumor, i.e. the expression and metabolic profiles, and the clinical metadata from the same patient may improve the understanding of breast cancer heterogeneity, and may lead to more patient specific treatment. In addition, this study may prove MR metabolomics to be a potential additional diagnostic tool for clinical use.

References: 1] Sorlie, T., et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci*, 2001; 98(19):10869-10874. 2] van't Veer, L., et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002; 415(6871): 530-536. 3] Parker, J.S., et al. Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes, *J Clin Oncol*, 2009; 27(8):1160-1167. 4] Moestue, S., et al. HR MAS MR Spectroscopy in Metabolic Characterization of Cancer, *Curr Top Med Chem*, 2011; 11(1):2-26. 5] Moestue, S., et al. Distinct choline metabolic profiles are associated with differences in gene expression for basal-like and luminal-like breast cancer xenograft models. *BMC Cancer*, 2010; 10:433. 6] Borgan, E., et al. Merging transcriptomics and metabolomics – advances in breast cancer profiling. *BMC Cancer*, 2010; 10:628.