Metabolic characterization of triple negative breast cancer

Guro Fanneløb Giskeødegård[1,2], Maria Dung Cao[1,2], Santosh Lamichhane[3], Beathe Sitter[4], Anna Bofin[5], Steinar Lundgren[2], Hans Fjøsne[2], and Tone Frost Bathen[1]
[1]*Dept. of Circulation and Medical Imaging, NTNU, Trondheim, Norway,* [2]*St. Olavs University Hospital, Trondheim, Norway,* [3]*Department of Food Science, Aarhus University, Denmark,* [4]*Sør-Trøndelag University College, Norway,* [5]*NTNU, Trondheim, Norway*

Target audience Basic scientists investigating metabolomic biomarkers for cancer diagnosis, prognosis and treatment.

Introduction and purpose Triple negative breast cancer (TNBC), characterized by estrogen receptor (ER), progesterone receptor (PgR) and HER2 negativity, represents 15-20% of all breast cancer cases. TNBC is associated with more aggressive and higher grade tumors and poor prognosis (1). Current treatment options for TNCB are few and the responses are often insufficient. Novel molecular targets for the treatment of TNBC are needed to improve the prognosis of this breast cancer subgroup. The purpose of this study was to characterize the metabolic patterns of TNBC as compared to triple positive breast cancer (TPBC), and to identify potential molecular markers of TNBC tumors for targeted treatment. The influence of individual receptors on the metabolic profile was also analyzed.

Methods Tumor biopsies from breast cancer patients (n = 73 patients (p)/106 biopsies (b)) were obtained during surgery. ER, PgR and HER2 status were determined by routine histopathology. The presence of cancer cells was evaluated by imprint cytology. The biopsies were analyzed by High Resolution Magic Angle Spinning MR Spectroscopy (HR MAS MRS) on a Bruker Avance III 600MHz/54 mm US. Spin-echo spectra (cpmgpr1d; Bruker) acquired with an echo-time of 273.5 ms were mean-centered and scaled by orthogonal signal correction (OSC), and analyzed by multivariate analysis using Partial Least Squares Discriminant Analysis (PLS-DA) with cross-validation (10% random subsets). Permutation testing was performed to evaluate the statistical significance of the classification results. Findings of important metabolites were confirmed by spectral integration of the metabolite peaks and the significance examined by Wilcoxon testing with Benjamini Hochberg correction for multiple testing.

Results The metabolic profiles of TNBC (n=19p/26b) were successfully discriminated from TPBC ($p < 0.001$) (Table 1, Fig. 1). The metabolic difference has contributions from ER, PgR and HER2 as positive status could be significantly separated from negative status in all three receptors independently (Table 1). Figure 2 shows the relative concentrations of metabolites between samples of different ER, HER2, and triple receptor status.

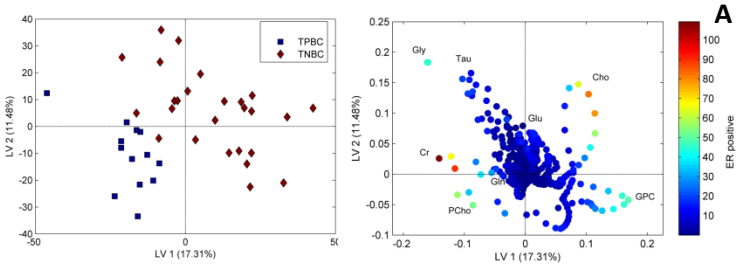

Figure 1: PLS-DA scores and loadings separating TNBP (red) from TPBC (blue)

Table 1: PLS-DA classification

	n (p/b)	Class. accuracy	p-value
TNBC vs triple positive	30/39	80.3 %	< 0.001
ER- vs ER+	73/106	74.3 %	< 0.001
PgR- vs ER+	73/106	67.0 %	< 0.001
HER2- vs HER2+	73/106	66.8 %	< 0.001

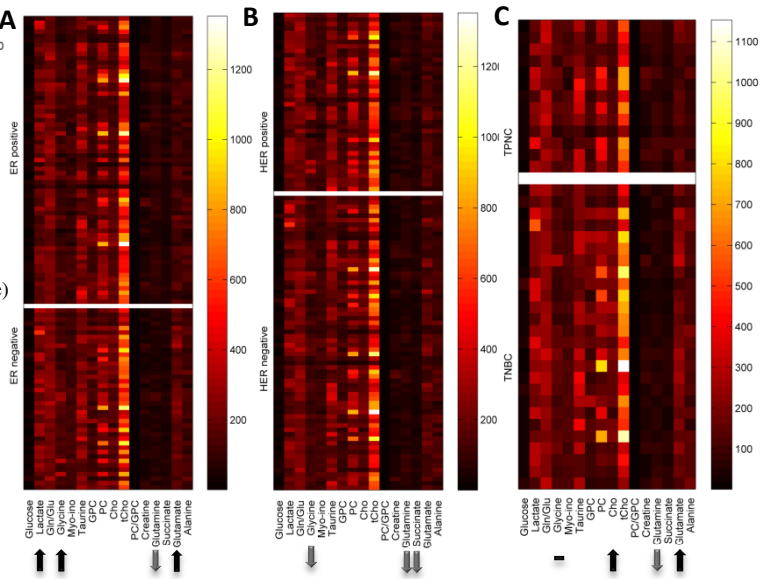

Figure 2: Relative metabolite concentrations. The arrows show metabolites with significantly higher (↑) or lower (↓) levels in ER negative, HER2 negative and TNBC in figures A, B and C, respectively.

Discussion TNBC has significantly higher choline levels compared to TPBC, which is in accordance with previous findings in the more aggressive basal-like xenografts and TNBC patients as compared to the less aggressive luminal-like xenografts and ER+/PgR+ breast cancer patients (2). In addition, TNBC has higher levels of glutamate and lower levels of glutamine. This may result from differences in energy metabolism as glutamine consumption has been shown to be closely correlated to glutamate release in cell culture (3). High levels of glycine have previously been shown to indicate a poor prognosis (4), possibly related to different proliferation rates (3). Glycine levels were higher in ER negative and HER2 positive tumors (Figure 2), cancelling out the significance of differences in glycine among TNBC and TPBC. Both ER negativity and HER2 positivity are associated with poor prognosis. Our results suggest different energy metabolism and proliferation in TNBC compared to TPBC. However, further studies on larger patient cohorts are needed to validate this hypothesis. We are currently working with further molecular characterization of TNBC.

Conclusion Our results reveal metabolic differences between TNBC and TPBC. These differences could help identifying targets for improved treatment of TNBC.

References (1) Brouckaert O, Int J Womens Health **4**, 2012 (2) Moestue et al, BMC Cancer **10**, 2010 (3) Jain et al, Science **336**, 2012 (4) Giskeødegård et al, NMR Biomed **25,** 2012.