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Comparative metabolomics of estrogen receptor positive and estrogen receptor negative breast cancer: alterations in glutamine and beta-alanine metabolism

Jan Budczies[a,b,*], Scarlet F. Brockmöller[c], Berit M. Müller[a], Dinesh K. Barupal[d], Christiane Richter-Ehrenstein[e], Anke Kleine-Tebbe[f], Julian L. Griffin[c], Matej Orešič[g], Manfred Dietel[a], Carsten Denkert[a,1], Oliver Fiehn[h,1]

[a]Institute of Pathology, Charité University Hospital, 10117 Berlin, Germany
[b]German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany
[c]Department of Biochemistry and Cambridge Systems Biology Centre, University of Cambridge, Cambridge CB2 1GA, United Kingdom
[d]International Agency for Research on Cancer (IARC), 69372 Lyon, France
[e]Interdisciplinary Breast Center, Charité University Hospital, 10117 Berlin, Germany
[f]Breast Center, DRK Kliniken Berlin, 12559 Berlin, Germany
[g]VTT Technical Research Centre of Finland, 02044 Espoo, Finland
[h]Genome Center, University of California Davis, Davis, CA, USA

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ABSTRACT

Molecular subtyping of breast cancer is necessary for therapy selection and mandatory for all breast cancer patients. Metabolic alterations are considered a hallmark of cancer and several metabolic drugs are currently being investigated in clinical trials. However, the dependence of metabolic alterations on breast cancer subtypes has not been investigated on -omics scale. Thus, 204 estrogen receptor positive (ER+) and 67 estrogen receptor negative (ER–) breast cancer tissues were investigated using GC–TOFMS based metabolomics. 19 metabolites were detected as altered in a predefined training set (2/3 of tumors) and could be validated in a predefined validation set (1/3 of tumors). The metabolite changes included increases in beta-alanine, 2-hydroyglutarate, glutamate, xanthine and decreases in glutamine in the ER– subtype. Beta-alanine demonstrated the strongest change between ER– and ER+ breast cancer (fold change = 2.4, p = 1.5E−20). In a correlation analysis with genome-wide expression data in a subcohort of 154 tumors, we found a strong negative correlation (Spearman R = −0.62) between beta-alanine and 4-aminobutyrate aminotransferase (ABAT). Immunohistological analysis confirmed down-regulation of the ABAT protein in ER– breast cancer. In a Kaplan–Meier

* *Corresponding author at:* Institute of Pathology, Charité University Hospital, Charitéplatz 1, 10117 Berlin, Germany. Tel.: +49 450 536 163; fax: +49 450 536 902.
E-mail addresses: jan.budczies@charite.de (J. Budczies), scarlet.brockmoeller@nnuh.nhs.uk (S.F. Brockmöller), berit.mueller@charite.de (B.M. Müller), barupal@gmail.com (D.K. Barupal), christiane.richter-ehrenstein@charite.de (C. Richter-Ehrenstein), a.kleine-tebbe@drk-kliniken-berlin.de (A. Kleine-Tebbe), mjlg40@cam.ac.uk (J.L. Griffin), matej.oresic@vtt.fi (M. Orešič), manfred.dietel@charite.de (M. Dietel), carsten.denkert@charite.de (C. Denkert), ofiehn@ucdavis.edu (O. Fiehn).
[1] Equal contribution.

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analysis of a large external expression data set, the ABAT transcript was demonstrated to be a positive prognostic marker for breast cancer (HR = 0.6, p=3.2E-15).

Biological significance
It is well-known for more than a decade that breast cancer exhibits distinct gene expression patterns depending on the molecular subtype defined by estrogen receptor (ER) and HER2 status. Here, we show that breast cancer exhibits distinct metabolomics patterns depending on ER status. Our observation supports the current view of ER+ breast cancer and ER– breast as different diseases requiring different treatment strategies.
Metabolic drugs for cancer including glutaminase inhibitors are currently under development and tested in clinical trials. We found glutamate enriched and glutamine reduced in ER– breast cancer compared to ER+ breast cancer and compared to normal breast tissues. Thus, metabolomics analysis highlights the ER– subtype as a preferential target for glutaminase inhibitors.
For the first time, we report on a regulation of beta-alanine catabolism in cancer. In breast cancer, ABAT transcript expression was variable and correlated with ER status. Low ABAT transcript expression was associated with low ABAT protein expression and high beta-alanine concentration. In a large external microarray cohort, low ABAT expression shortened recurrence-free survival in breast cancer, ER+ breast cancer and ER– breast cancer.

1. Introduction

During the recent decades it has become clear that breast cancer can no longer be considered as a single disease entity. Molecular subtypes can be defined based on estrogen receptor (ER), progesterone receptor (PgR) and HER2 protein expressions or microarray-measured gene expression patterns [1–3]. As predictive markers for the response to adjuvant anti-hormonal and adjuvant anti-HER2 therapy, immunohistological determination of hormone receptor and HER2 status is a worldwide standard in the histopathological evaluation of breast cancer tissues. In the 2011 St. Gallen Expert Consensus this standard was expanded by Ki67 immunohistochemistry to separate the low-risk luminal A subtype from the high-risk luminal B subtype [4]. Molecular subtyping is essential for each breast cancer patient, because it is linked to prognosis [5] and necessary for therapy selection [4].

Complementary to viewing cancer as a genetic disease, cancer can be considered as a metabolic disease. This standpoint has a long history starting with Warburg's seminal work [6] and continues to raise interest in recent years [7–10]. While mutations in oncogenes and tumor suppressor genes are believed to constitute the ultimate cause of malignant growth, metabolic changes are the final answers of the cell to genetic changes and are directly linked to cell physiology. Many of the metabolic alterations can be targeted therapeutically [11] and metabolic drugs targeting glycolysis, glutaminolysis, fatty acid synthesis, hypoxia-inducible factor (HIF) signaling and other pathways are currently being investigated in clinical trials [12]. Therefore, to define patient populations that profit most strongly from a metabolic drug, it is important to analyze the spectrum of metabolic changes in the different subtypes of breast cancer.

In the past, the main domain of cancer metabolomics was to investigate blood serum or urine samples. This approach was employed for the detection of primary breast cancer [13] and recurrent breast cancer [14]. The number of metabolomics studies investigating cancer tissues is more limited. Recently, in two studies [15,16] breast cancer tissues were investigated using high-resolution magic angle spinning magnetic resonance (HR-MAS MR). In two preceding studies in ovarian cancer and in colorectal cancer, we established the use of gas chromatography combined with time of flight mass spectrometry (GC–TOFMS) based metabolomics for the investigation of the metabolic changes in cancer tissues [17,18]. We have also generated a molecular map of the metabolic changes in breast cancer compared to normal breast tissues and showed that breast cancer and normal tissues can be separated with high sensitivity and specificity using GC–TOFMS [19].

In the preceding breast cancer study [19], we analyzed the metabolic changes occurring after the malignant transformation, but we did not investigate differences between the molecular subtypes of breast cancer. The aim of this work is to investigate the differences in central metabolism between ER– and ER+ breast cancer. To this end, a large cohort of breast cancer tissues was investigated using GC–TOFMS based metabolomics, whole-genome expression analysis and in-situ analysis of the ABAT protein.

2. Material and methods

2.1. Study cohort

The study cohort consisted of 271 breast cancer tissues (204 ER+ and 67 ER– tumor tissues) from a biobank at the Pathology Department of Charité Hospital (Suppl. material 1). The project was approved by the ethics board of the Charité Hospital (Reference number EA1/139/05 Amendment 2008). Samples were included after passing histopathological quality control confirming ≥40% tumor area. The experimental design included predefined splitting of the tissues in training set (TS, 2/3 of tumor) and a validation set (VS, 1/3 of tissues) having similar clinicopathological characteristics. In detail, tumor samples were split randomly in such a way that the training (2/3) and validation (1/3) sets did not differ with respect to the following parameters: tumor stage, tumor grade, age (≤50 and >50 years) and estrogen receptor status. To this end, 100,000 splits were randomly drawn and the one with the lowest