Targeted Metabolomics Analysis of Plasma and Tissue samples of Women with Breast Cancer

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Introduction

Targeted metabolomics in cancer research offers the unique opportunity for direct characterization of animal models on a functional level, thus gaining new knowledge of the pathophysiology as a fundament for biomarker discovery and validation for breast cancer diagnosis. Breast cancer (BC) is the most common cancer in western women affecting annually 0.6 million people. The current therapies are based on clinical parameters such as pathological evaluations of tumour size, lymph node as well as hormone and HER2 receptors status. Additionally, some markers for BC diagnosis and treatment evaluation are routinely used. Despite the progress, these measurements made during the past years, still 0.2 million of women die per year from breast cancer. In order to advance the methodological repertoire for cancer diagnosis and follow-up, the multi-platform EU-project COBRED was started in 2005. Part of the project included the metabolic characterization of plasma and tissue samples from more than 100 BC patients. In the framework of this project, sample work-up procedures and analytical platform efficiencies were optimized to increase data quality. Moreover, the spectrum of detectable metabolites was broadened to allow the discovery of new biomarker candidates.

Method and Materials

Tissue homogenization:

- Weigh sample into pre-cooled Precellys tubes with various sized ceramic beads
- Extract samples by addition of a mixture of ethanol/phosphate buffer 85:15 (v/v) at a ratio of 1:3 (v/v)
- Centrifuge samples (18,000 x g, 2 °C, 5 min)
- Pipette onto filter plates

Method Parameters:

- Only 10 μl sample volume (FIA-MS/MS)
- Only 20 μl sample volume (LC-MS/MS)

Sample Preparation:

- Protein precipitation in 96-well plate format in combination with PItC-Derivatization for:
  - Lids, Acylcarnitines, Hexose (190 MRM, FIA, method 1)
  - Amino acids and Biogenic amines (62 MRM, LC, method 2)
  - Bile acids (17 MRM, LC, method 3, see chromatograms)

HPLC/MS/MS:

- HPLC: Agilent System 1200
- -TLC-PAL AS
- Column: Agilent XDB C18
- ESI-API 4000 QTRap MS/MS
- Positive / Negative MRM mode

Results Tissue

- Figure 1. Box plots showing the significantly different content of: PC aa C42:1, PC aa C42:2 and PC and PC aa C42:4 (upper panel, left, middle and right) and PC ae C42:3 and PC ae C42:4 (lower panel, left, middle and right); in solid tumour tissues. Tumour tissues were divided in Triple Negative (TN, no receptors over-expression), Her2+ (Her2+ receptor over-expression), and others (Estrogen and/or Progesterone receptors positive).

Figure 2. Box plots showing the significantly different content of: Sarcosine, Taurine and Spermine in the solid tumour tissues (tumour) compared to surrounding healthy tissues (surrounding).

- Figure 3. Compound class specific heatmap representation of the actual metabolite content of Amino Acids in tissue. Measurements greater (resp. lower) than the analyte average concentration are displayed in red (resp. blue) and undetected signals are colored in grey.

- Figure 4a. Relative changes after surgery in plasma concentrations (log2 transformed) of the middle-long chain acylcarnitines. The breast cancer patients were grouped according to the tumor size.

- Figure 4b. Relative changes after surgery in plasma concentrations (log2 transformed) of the phosphatidylcholines. The breast cancer patients were grouped according to the nodes status.

- Figure 5. Box plots showing the time course of Arachidonic acid (left panel) and sphingomyelin C20:2 (right panel) concentrations.

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Conclusions

- In total, 219 metabolites of 6 biological relevant compound classes were quantified using 30 ISTDs to study the sample work-up conditions of tissue and plasma.
- ETOH/PB gives best compromise in terms of CVs, controllable matrix effect and sample handling.
- This targeted metabolomics approach allows:
  - to distinguish BC molecular subtypes
  - to clearly differentiate between tumour and healthy surrounding tissue
- Effects of tumour removal and chemotherapy could be monitored in plasma samples in a follow-up study.
- Our data could further form the basis of a standardized work-up protocol to perform absolute quantitative metabolomics in tissue and plasma of BC patients in order to improve the early detection and learn more about cancer specific metabolism.

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