qPCR gene expression profiles in peripheral blood mononuclear cells of breast cancer patients

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Introduction
Breast cancer is a disease with highest incidence in women worldwide. The incidence has been rising in last decades and nowadays it ranges up to 90 per 100 thousands in developed countries (1). Despite the genetic background and the disease itself are very well studied, there is still regular lack of sufficient and reliable biomarkers.

In this study, we performed qPCR gene expression profiling in the patients group (n = 64) compared with the group of healthy donors (n = 20). The patients group proportionally consisted of primary as well as metastatic breast cancer patients. PBMCs were collected from peripheral blood using Ficoll-Paque, followed with appropriate RNA isolation and cDNA synthesis. The gene panel consisting of 45 breast cancer associated genes, previously established in TATAA-Biocenter was assessed using 384 well qPCR platform Viia7. Data analysis was carried out using Genex Professional software and revealed 15 genes to be significantly differentially expressed in the patients group compared to healthy donors.

(1) http://www.cancerresearchuk.org/cancer-info/cancerstats/world/breast-cancer-world/

Aim of study
The aim of the study is to identify qPCR gene expression profiles of peripheral blood mononuclear cells of breast cancer patients that differ from healthy controls.

Materials and methods

Patients
The patients group (n = 64) was divided into two subgroups. Subgroup 1 consisted of 32 patients in disease stage I and II. None of the subgroup I patients received neoadjuvant chemotherapy prior to the inclusion into the study. Subgroup 2 consisted of 32 patients in stage III and these patients had undergone neoadjuvant chemotherapy. The data were compared with the Control group of healthy donors (n = 20).

Methods
PBMCs were collected from 8 ml of peripheral blood using Ficoll-Paque density gradient solution (GE Healthcare, USA). Cell lysis of appropriate amount of cells (from 3 to 5 x 10⁶ cells) was prepared in 350 µl of RLT buffer followed with total RNA extraction (RNA Easy Mini Kit, Qiagen, Germany). RNA concentrations were determined with NanoDrop 2000c UV-Vis spectrophotometer (Thermo Scientific, Germany), and RNA integrity was assessed using Agilent 2100 Bioanalyzer (Agilent, USA). Appropriate amount of cDNA was synthesized from 100 ng of total RNA using iScript cDNA synthesis kit (BioRad, USA). TATAA Universal RNA Spike was used for quality control of reverse transcription reaction, to exclude any enzymatic inhibition.

For expression profiling itself, the gene panel consisting of 45 breast cancer associated genes previously established in TATAA-Biocenter was assessed in 10 µl reactions using TATAA SYBR® GrandMaster® Mix and 384 well qPCR platform Viia7. Data analysis was carried out using Genex Professional software (MultiD, Sweden).

Results

Fig. 1A: Principle Component Analysis (PC1/PC2)

Fig. 1B: Principle component analysis (PC1/PC2/PC3)

3D variant of the Principle component analysis (PC1/PC2/PC3) confirmed the distribution showed in the panel 1A (PC1/PC2). Patient group is marked in red, control group is marked in blue.

Fig. 2: Volcano Plot

Volcano plot is based on the univariate analysis. It shows 15 genes significantly differentially expressed (with low p-value) in the group of Patients vs. the Control group.

Fig. 3: Differential expression Plot

Differential Expression of 15 genes in the Patient group (red) vs. the Control group (blue).

Fig. 4: Heat Map Analysis

Conclusions and Future plans

Using a Genex Software, the data analysis revealed 15 genes to be significantly differentially expressed in the patients group compared to healthy donors. 6 genes were found to be upregulated in patients and 9 genes were identified to be downregulated in patients’ PBMCs. The effect of chemotherapy itself was excluded.

Our future plans are to enroll more breast cancer patients into this study, establish an additional Control group of 20 ovarian cancer patients and this way validate and establish the importance of 15 selected genes to be the potential markers of the breast cancer.

Acknowledgements
This work is supported by Sotio, a.s. and grants IGA NT 11404-5 and GA UK 573 412.