HTG Molecular

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AG13.03 - Targeted mRNA sequencing using small formalin-fixed, paraffin-embedded core biopsies to evaluate immune- and tumor-associated gene expression in breast cancer

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Background

Immunomodulatory processes play a crucial role in the biology of breast cancer and are of particular interest in the context of emerging immunomodulatory therapies like PD-L1 inhibition. In this study, we evaluated the use of a system for targeted mRNA sequencing (RNA-Seq) for the use on small, formalin-fixed and paraffin-embedded (FFPE) core biopsies to establish a platform for future tumor-immunological studies in the context of clinical trials.

Methods

53 pre-therapeutic core biopsies of breast cancer patients that received treatment within the neoadjuvant GeparQuattro trial were used in this study. We prepared 12 mm^2 of tissue on a 5 µm thick unstained paraffin slide and processed the sample on a HTG EdgeSeq Instrument (HTG Molecular Diagnostics, Tucson, AZ, USA) using HTG EdgeSeq Oncology Biomarker Panel to capture the expression of 2560 transcripts and RNA sequencing (Illumina NextSeq). We repeated full technical replicates of eight samples at the Charité. For alignment, we used the HTG EdgeSeq Parser, scale-normalized the data and calculated log2-counts-per-million (edgeR bioconductor package). For thirteen mostly immune-associated genes, additional quantitative PCR (qPCR) data were available for 41 cases.

Results

Library preparation was successful in 48 of 50 samples, 45 could be successfully sequenced. Correlation coefficients for the comparison of the two platforms (RNASeq and qPCR) ranged between 0.243 and 0.959 with coefficients above 0.6 in eight of 13 genes. Values of ESR1 and ERBB2 were concordant with qPCR-based classification ($p < 0.001$). Unsupervised hierarchical clustering yielded two clusters according to hormone receptor status and differential gene expression analysis resulted in known targets of ESR1. The technical and inter-laboratory reproducibility was good with $\rho = 0.665 - 0.887$ for the eight replicates.

Conclusion

The HTG EdgeSeq system is applicable to small FFPE core biopsies. Overall, there was a good concordance between RNA-Seq and qPCR data and a good technical reproducibility. The strength of the biological signal, especially its association with clinical endpoints, requires further study.