Transcriptomic analysis of circulating tumor cells in metastatic breast cancer: the new frontiers of liquid biopsy for personalized medicine

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Background & objectives

Circulating tumor cells (CTCs) are a type of liquid biopsy capable of recapitulating the full cancer biological system, therefore representing a promising real-time biological readout of disease evolution and treatment resistance. Here, we aim at investigating the transcriptomic profile of CTCs from nine luminal metastatic breast cancer (MBC) patients at single-cell level and to identify gene expression signatures associated with clinical parameters.

Methods

CTCs were enriched from peripheral blood using RosetteSep CTC Enrichment cocktail containing anti-CD36, then incubated with antibodies targeting epithelial (EpCAM, E-cadherin) and leukocyte (CD45) markers. Hoechst33342 was used for nuclear staining. DEPArray NxT was used for phenotypic analysis and live single-cell recovery. Libraries were prepared using QIAseq UPX 3' Transcriptome kit and sequenced on Illumina MiSeq. CLC Genomics Workbench was used for bioinformatic and statistical analysis. Enrichr was used for enrichment analysis on datasets: Wikipathways, Gene Ontology (GO)-Biological Processes (GO-BP) and -Molecular Function (GO-MF).

Results

A median of 9 CTCs (ranging 2-31) were found in 7/9 patients (77,8%). CTCs (n=13) from 2 patients with progressive disease (PD) compared to CTCs (n=24) from 5 non-PD patients displayed overexpression of genes associated with BC progression (*NEAT1*, *BRD4),* while genes involved DNA break-repair were downregulated (*BRCA2*, *XRCC5*, *HMGB1*), regardless of metastasis site and time-point. Enriched terms in CTCs from PD patients, were associated with regulation of apoptotic process (GO-BP) and kinase binding (GO-MF). CTCs (n=15) from 3 patients with bone metastasis compared to CTCs (n=22) from 4 patients with metastasis at other sites harboured overexpression of genes reported to be involved in improved migration (*CDC42*, *ARF6*, *TMSB10*) as well as in metastatic

osteotropism (*S100A4*, *VAPA*). Terms associated with regulation of adhesion-dependent cell spreading (GO-BP) and VEGFA-VEGFR2 signaling pathway (Wikipathways) emerged as enriched in BM patients' CTCs.

Conclusion

Our results, although hypothesis generating, suggested the potential of CTCs- based transcriptomics in MBC patients for a granular real-time characterization providing new insights on the clinical- biological evolution of MBC and its underlying mechanisms of metastatic cascade and organotropism. Once confirmed on a large case series, this type of investigation would represent an important approach to improve personalized medicine.