Development of a protocol for isolation and gene expression analysis in single circulating tumour cells

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Introduction

Circulating tumour cells (CTCs) are malignant tumour cells which detached from the primary tumour and circulate in peripheral blood (Thiery, Acloque, Huang, & Nieto, 2009). Recent discoveries show that classical epithelial CTCs can undergo epithelial-mesenchymal transition, which changes their phenotype to mesenchymal (Cichon, Nelson, & Radisky, 2015; Thiery et al., 2009). Mesenchymal CTCs can acquire additional malignant properties. Due to their rarity and heterogeneity CTCs can be best characterized on the level of single cell (Micalizzi, Haber, & Maheswaran, 2017; Palmirotta et al., 2017).

Materials and Methods

The model of epithelial and mesenchymal breast cancer CTCs was MCF-7 and MDA-MB-231 breast cancer cell line, respectively. To mimic clinical samples, cell lines were spiked into peripheral blood obtained from healthy donors. Blood sample was separated by density gradient centrifugation, then mononuclear fraction containing CTCs was collected and immunofluorescent labelling was performed for detection of EpCAM (epithelial marker), cell surface Vimentin (mesenchymal marker) and CD45 (common leukocyte antigen). Next, depletion of blood leukocytes was performed with immunomagnetic beads. Cells positive for EpCAM or Vimentin, but negative for CD45, were picked using micromanipulatory isolation. Single cells were processed according to the modified SMART-Seq2 protocol to allow RNA isolation, reverse transcription and cDNA preamplification. Gene expression analysis was performed by qPCR in order to test reference genes and genes associated with epithelial and mesenchymal phenotype.

Results

Protocol for CTCs isolation was improved and optimized. In single epithelial MCF-7 cells higher level of transcripts associated with the epithelial phenotype (EpCAM, E-cadherin, $p=0.04$ and $p=0.0002$, respectively) was detected in comparison to mesenchymal cells. Whereas in single mesenchymal MDA-MB-231 cells, higher level of transcripts associated with mesenchymal phenotype was found (vimentin $p=0.00008$). Additionally, some variability in gene expression in single cells was observed, but it

was smaller than the variability between epithelial and mesenchymal phenotype.

Discussion

According to the literature, the presence of CTCs has a significant role in tumor dissemination, invasion and metastasis (Palmirotta et al., 2017). In 2017, Chen et al. published a report about the use of the Smart-seq method in terms of tumor cell profiling in early breast cancer patients (Chen et al., 2013). The analysis of single CTCs transcriptomes was carried out in the case of lung cancer ((Chen et al., 2013), pancreas (Lapin et al., 2017). However, there are no studies which investigate a single CTCs in the clinical approach to breast cancer. The improved method allows to detect single cells with epithelial and mesenchymal phenotype, isolate them and analyze their gene expression profile. CTCs obtained from breast cancer patients in this way can be classified in terms of the epithelial-mesenchymal character which, according to the literature, is associated with different patients' prognosis (Aceto, Toner, Maheswaran, & Haber, 2015; Ksiazkiewicz, Markiewicz, & Zaczek, 2012).

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