ULTRA-HIGH PURITY CAPTURE OF CIRCULATING TUMOR CELLS AND GENE MUTATIONS DETECTION

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ABSTRACT

This paper reports on the detection of gene mutation in circulating tumor cells (CTC) captured using the Ephesia microdevice. In an earlier work, we have demonstrated the advantages of the Ephesia system to capture rare CTCs in blood samples obtained from metastatic cancer patients [1]. The device combines the advantages of both microfluidics and immunocapture to achieve high purity (below 0.01% contamination) and high capture efficiency (> 90% of EpCAM-positive cells). After an initial proof of concept using cells lines, the Ephesia system has recently been compared to the Veridex Cellsearch and validated with real patient samples. In this work, we show the possibility to release the captured cells and to perform a subsequent genomic analysis. The microchip design and analysis workflow was optimized to provide a capture purity which is compatible with conventional PCR.

KEYWORDS: Circulating Tumor Cells, CTCs, Rare cell capture, Immunocapture, Genomic analysis

INTRODUCTION

It is now well admitted that metastatic development in distant organs is caused by circulating tumor cells (CTCs). Circulating tumor cells are defined as malignant cells issued from the primary tumor and escaping in the blood through an extravasation process. They are currently considered as relevant biomarkers for cancer and there is today a strong interest in characterizing circulating tumor cells (CTCs), as easy and minimally invasive « liquid biopsies ». They should provide, during the « blind » period following primary treatment, a powerful tool to evaluate the risk of metastatic development, to follow in real time the efficiency of treatments, identify « therapeutic escapes », and guide clinicians in the prescription of alternative treatments. Besides the CTCs count, there is a strong interest in collecting additional molecular information to better orient the patient treatment. In particular, proteomics and genomics analysis on CTCs [2] are critical to help clinicians in their choice, to better understand the metastatic process, and to give insight in the evolution of the disease. Among the different techniques available, the possibility of detecting mutations in the genome of those cells is of great interest in particular in the context of personalized medicine. However, the detection of CTC mutations is usually made difficult due to the high level of contamination by healthy blood cells.

EXPERIMENTAL

We have recently developed a microfluidic platform devoted to CTC immunocapture. The so-called Ephesia technology [1] involves the formation, in a microchannel, of a regular array of capture columns made of antibody-coated magnetic beads directed against CTCs [3]. The design of the microfluidic chip is described and characterized in [4]. Briefly, the Ephesia technology uses the spontaneous self-assembly property of superparamagnetic beads to create spontaneously regular and high aspect ratio columns. These columns were integrated in PDMS microdevices and anchored on self-assembled magnetic patterns. The fabrication process thus avoids the need of costly and complex microfabrication technology. Moreover, all the delicate biofunctionalization steps required to prepare libraries of microparticles can be performed in batch outside the system, thus reducing cost and increasing reproducibility. In this method, the magnetic particles are first injected in the device, then immobilized in the chip using an external magnetic field, and finally the cells are flowed through. This approach presents two major advantages: first, the number of particles required is proportional essentially to the number of cells to capture and not to the total volume of the sample (as in batch methods). Second, the interaction between the cells and the immobilized particles is not induced by Brownian motion, but is controlled by the hydrodynamic flow inside the array. In addition, the system provide a fully automated platform to perform of all the staining and observation protocols currently used in optical cytology. For that purpose, a MFCS Flow Controller (Fluigent) was used to adjust the flow rates and to perform the automation of the reagents and samples injection sequence in the device.

RESULTS AND DISCUSSION

We first showed first the ability of the Ephesia device to capture cells from breast cancer patient samples. The obtained results were compared with the gold-standard and FDA approved method Cellsearch from Veridex (fig 1). Experiments were carried out on breast cancer samples. We showed that the results achieved with the Ephesia chip on

real patient samples are consistent with Cellsearch device and offer, in some cases, a higher capture efficiency. We can therefore consider that the EPHESIA system is compatible with clinical analysis.

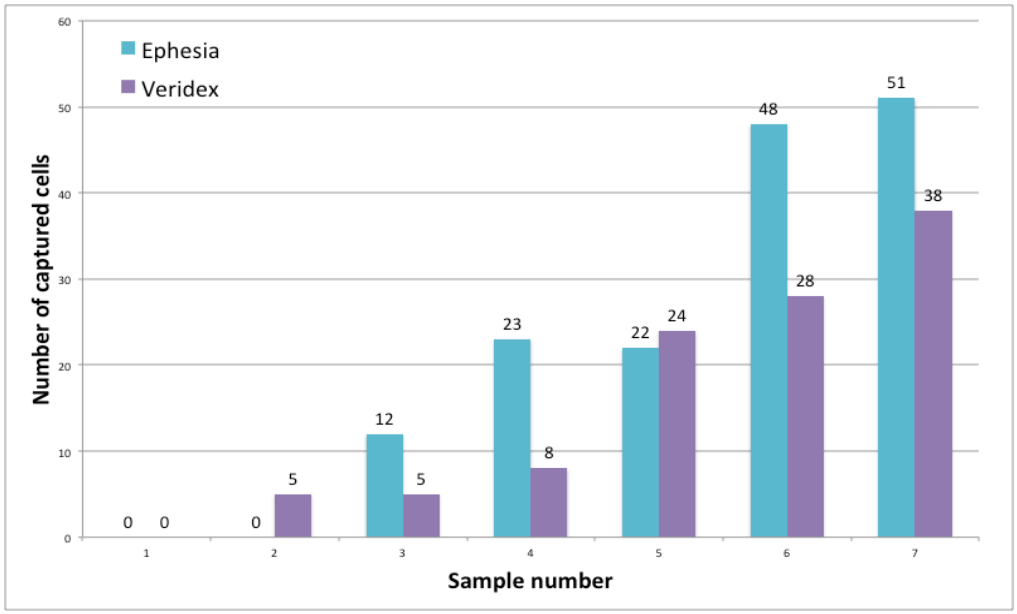

Figure 1: Results obtained on capture of CTCs from breast cancer patients and comparison with the Veridex analysis platform

Based on the workflow presented in fig. 2, we investigated the opportunity to go beyond CTC counting and to benefit of high purity achieved with EPHESIA for further molecular analysis (contamination ratio below 10 cells for 1 CTC). These performances make the Ephesia system appealing for highly sensitive PCR methods such as digital PCR. It should also reach the threshold of standard qPCR thus making the mutation detection achievable in routine hospital diagnosis laboratory.

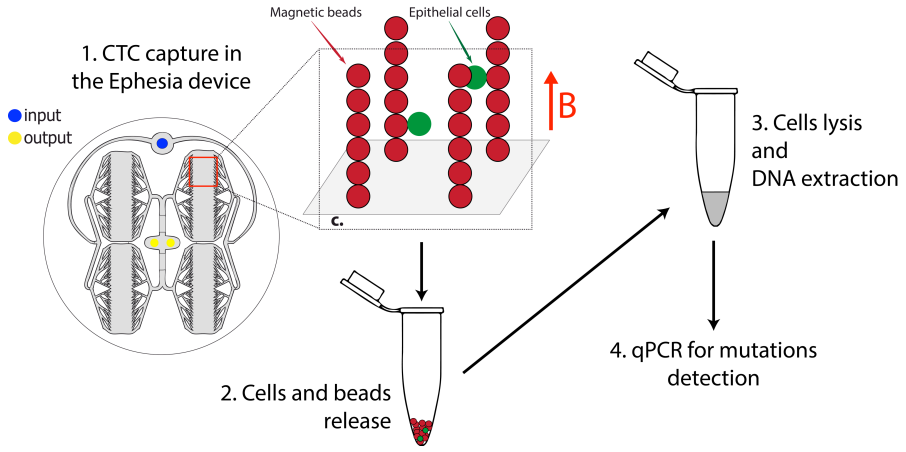

Figure 2: CTC capture and analysis workflow

We focused our attention on the potential detection of mutations in cells after their capture in the chip. As a target, we focused on the detection of the PIK3CA gene. PI3Ks are lipid kinases that are important in controlling signaling pathways involved in cell proliferation, cell death, motility and cell invasion. We first investigated the detection of the PI3KCA mutation in CTCs model cells line (MCF7) in pure samples or in presence of contaminating cells in different ratio. Different concentrations of MCF7 cells (from 20 to 2000) were spiked in PBS or blood and processed through the chip. After nucleus staining and counting, the whole chip was flushed (by releasing the magnetic field) with PBS and cells and beads were collected for further analysis. DNA extraction was achieved off-chip and conventional quantitative PCR was performed. Results obtained for 12, 128 and 1250 MCF7 cells spiked in PBS are presented in figure 3. We showed that thanks to its high selectivity and specificity, the Ephesia technology could be used as a enrichment step for further molecular analysis of the captured CTCs (by immunostainings), followed by a post-capture mutation detection.

Using standard molecular biology protocols the detection of PI3KCA mutation was made possible even for very low cell counts. Experiments performed with cells spiked into blood showed similar results with a shift off the CT value of 0.5 (N=1).

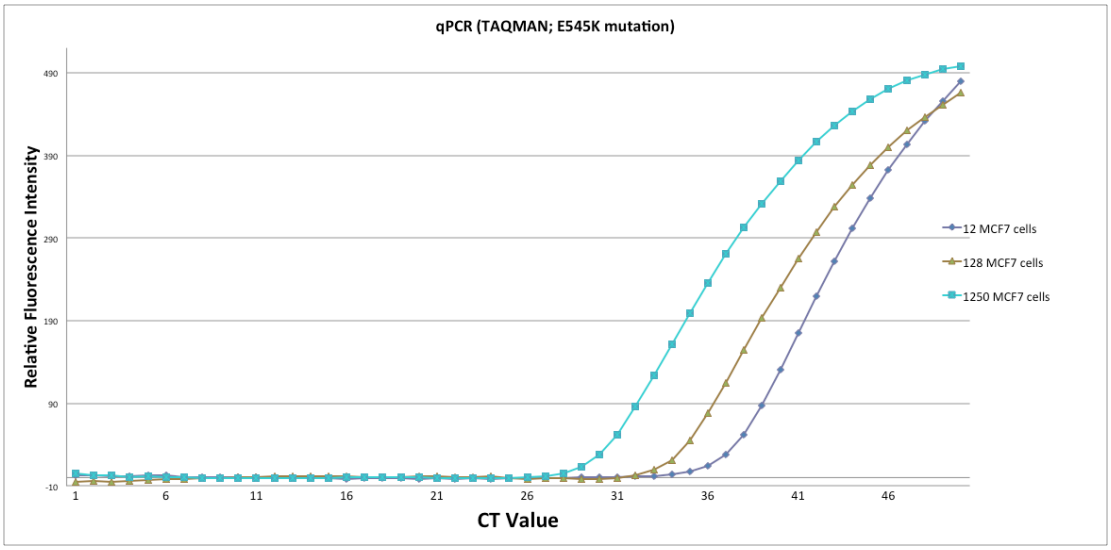

Figure 3: PI3K mutation detection obtained by qPCR on MCF7 (breast cancer) cell lines. The curves were obtained for 12, 128 and 1250 captured cells on Ephesia microdevice

CONCLUSION

In this work we have shown that the EPHESIA technology provides capture performances that are comparable or better to those of Veridex. We believe that this technology offers additional qualitative advantages. In addition to the possibility of operating the CTC analysis in a fully automated manner the specificity and purity resulting from the immunocapture allows the implementation of genetic typing using conventional qPCR methods. The demonstration of the PI3KCA mutation detection in blood samples illustrates the potential performances and interest of the Ephesia strategy for a routine analysis in a clinical environment.

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