A fast and sensitive workflow to screen therapy-relevant mutations in circulating tumor cells and quantification of cancer-associated exosomal mRNA in plasma of cancer patients

Martin H.D. Neumann, Nina Kessler, Sebastian Bender, Thomas Krahn, Thomas Schlange
Bayer AG, Pharmaceutical Division, Wuppertal/Berlin, Germany

INTRODUCTION

• The tests analyzing rare circulating cells or nucleic acids in blood are considerably hampered by liquid biopsies.
• Liquid biopsies offer a simple, fast, and cost-efficient way to monitor the status of a disease or the efficacy of a treatment at any time point with acceptable risk and minimal invasiveness.
• The detection requires robust, highly sensitive technologies like droplet digital PCR (ddPCR) as the volumes of available samples can be limited and rare cells as well as circulating nucleic acids are often present in low abundance.

• Here, we present a novel workflow combining effective enrichment of rare cells with the microfluidic filtration device Parsortix™ and the detection of point mutations by ddPCR (Figure 1). The approach enables the analysis of therapy-relevant point mutations and the quantification of circulating tumor cells (CTCs) as well as extracellular vesicle-derived mRNA (EVmRNA).

CONCLUSIONS

• As a screening approach, ddPCR allows the verification of therapy-relevant point mutations in CTCs. The sensitivity of point mutation determination by ddPCR is near to single cell level and in combination with the CTC counting, genetic information and CTC counts can be combined.
• Extracellular vesicles and especially exosomes, transporting small amounts of RNA, proteins and other factors, serve as a biomarker platform.
• Here, we show that EVmRNA can reflect treatment effect by showing an increase or decrease of target gene expression detectable by ddPCR. Moreover, EVmRNA may reflect the expression profile of cancer cells, but this is dependent on the target. EVmRNA could also be successfully detected and quantified in human plasma.
• A larger cohort and a deeper investigation on cell-based assays will follow to support these findings.

ACKNOWLEDGEMENTS

This work was supported by the Innovative Medicines Initiative Joint Undertaking under Grant Agreement n° 115749, resources of which are composed of financial contribution from the European Union’s Seventh Framework Programme (FP7/2007-2013) and EFPIA. Samples from patients and healthy volunteers, respectively, were collected under signed informed consent. Aurexel Life Sciences Ltd. (www.aurexel.com) is thanked for editorial assistance in the preparation of this poster, funded by Bayer AG.

DO NOT POST

A fast approach for screening point mutations in rare cells

The novel workflow enabled the quantification of low numbers of circulating tumor cells (CTCs) and their analysis for therapy-relevant point mutations (Figure 2). The KRAS G12V and TP53 R158L mutations were detected at an expected range (Figure 2B), demonstrating that in-cassette staining, cell quantification and mutation analysis are applicable methods in the screening approach.

Results

A fast approach for screening point mutations in rare cells

A novel workflow enabled the quantification of low numbers of circulating tumor cells (CTCs) and their analysis for therapy-relevant point mutations. The workflow, demonstrated in Figure 2B, showing that in-cassette staining, cell quantification and mutation analysis are applicable methods in the screening approach.

Figure 1. Workflow from a liquid biopsy to quantification of rare cells or nucleic acids. A simple, fast and sensitive workflow to screen therapy-relevant mutations in circulating tumor cells and quantification of circulating nucleic acids are often present in low abundance.

Figure 2. Detection of point mutations in cancer cells after rare cell enrichment. Non-small cell lung cancer (NSCLC) H441 (KRAS mutant) or breast cancer cell line MCF-7 (TP53 mutant) were spiked into healthy plasma. A simple, fast and sensitive workflow to screen therapy-relevant mutations in circulating tumor cells and quantification of circulating nucleic acids are often present in low abundance.

Extracellular vesicles (EV) are actively secreted by cells, including cancer cells, and their cargo contains, amongst other RNA. To determine if EVmRNA can be used to monitor pharmacological effects, the amount of cancer-associated EVmRNA secreted by the cells after cisplatin treatment was quantified.

Quantitative analysis of vesicle-derived mRNA reflected the therapeutic effect and provided implications of mRNA expression in cancer cells (Figure 3).

In the blood of breast cancer patients, the HER2 level detected in EVs reflected the HER2 status of the primary tumor (Figure 3D).

Figure 3. Detection of extracellular vesicle-derived mRNA (EVmRNA) in cancer cells. H1563 and NCI-H1573 (H1573) NSCLC cells were treated with 50 µM cisplatin for 24 h. The supematant and cells fractions were collected and EVmRNA expression was performed.

All H1573 cells were found to be more resistant to cisplatin treatment than H1563 cells (percentage of dead cells: 18.79 and 77.41%, respectively, compared to a non-treated control).

B. Changes in gene expression in all H1563 and H1573 cells (WBC or cellular fraction) with or without cisplatin treatment as detected by ddPCR. Altered mRNA expression in response to cisplatin treatment was observed for example for FOS in both the EVs and the cellular fraction. HER2 and SF3B1 showed downregulation upon treatment. No changes were observed for ACTB (baseline gene). FOSL1 showed significant changes in the cellular fraction, but not in the EV fraction. The experiments were performed with three independent biological replicates.

C. To determine if a multiplex target quantification is possible, a PanCancer Immune Profiling assay (NanoString) was performed. Only target genes which were 1.5-fold above background and showing a 2-fold differential expression compared to the control were shown. Some genes went up or downregulated by up to 10-20 times.

D. EVmRNA profiling of PARP, EPCAM and HER2 in plasma samples from patients with breast cancer or NSCLC, as well as from healthy volunteers (n=3). The HER2 levels were assessed specifically in cancer specimens while these targets were not detected in plasma samples from healthy donors.

Expression (copies/µL) ± SD

Figure 4. Expression of various genes in EV from H1563 and H1573 cells as quantified by ddPCR. Genes were selected for analysis based on their expression above background and showing a 2-fold differential expression compared to the control are shown. Some genes were up- or downregulated (e.g. up: FOSL1, down: EPCAM) and were included in a multiplex analysis.

EVmRNA profiling of PARP, EPCAM and HER2 in plasma samples from patients with breast cancer or NSCLC, as well as from healthy volunteers (n=3). The HER2 levels were assessed specifically in cancer specimens while these targets were not detected in plasma samples from healthy donors.