Targeted mutation detection in individual circulating tumor cells using a modified UltraSEEK® workflow for the MassARRAY® System

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Background & objectives

The very low amount of DNA obtained from single circulating tumor cells (CTCs) usually requires a whole genome amplification (WGA) step that complicates the workflow for assessing the mutational status of CTCs and possibly adds a technical bias. We sought to establish a straightforward protocol for detection of a panel of pre-defined mutations on a single-cell level which would circumvent the need for WGA. For this purpose, a pre-existing targeted PCR reliant ctDNA profiling assay (UltraSEEK® Panel) was expanded by an additional PCR to be tested in different single-cell settings and on CTCs from patients. From a cohort of 32 metastatic melanoma patients, CTCs were analyzed with the adapted UltraSEEK® procedure while ctDNA was analyzed with the UltraSEEK® procedure as recommended by manufacturer's instructions.

Methods

The resulting workflow was initially verified on low input of genomic DNA (7 pg) and single tumor cells derived from SK-MEL-28 (BRAF V600E), SK-MEL-30 (NRAS Q61K), SK-MEL-2 (NRAS Q61R), WM1366 (NRAS Q61L) melanoma cell lines. Additionally, to mimic real patients' samples, these tumor cells were spiked into healthy donors' blood samples and captured using either Parsortix® or Cellsearch® systems.

Results

Four different mutations from the UltraSEEK® Melanoma Panel were validated on cell culture level, with global mutation detection being 83% in cell culture cells isolated directly from cell culture, 75% in cells isolated with Parsortix® and 84% in cells isolated with Cellsearch®. In our cohort, 69 % (22/32) of the patients had at least 1 CTC and a total of 130 CTCs could be analyzed. Twenty-five different mutations were detected among CTCs, with 70 CTCs harboring only 1 mutation and 6 CTCs harboring multiple mutations. 61% (19/31) of the patients had at least 1 mutation detected on ctDNA level, with a total of 10 different mutations being discovered. 47% (8/17) of the matching ctDNA and CTC samples shared at least 1 common mutation.

Conclusion

We have established an innovative and easy-to-handle way of testing individual CTCs for mutations without the need for WGA, which will aid further studies investigating intra- and inter-tumoral heterogeneity of CTCs (Keller and Pantel, Nature Rev. Cancer, 2019).