Detection of circulating tumor cells from a multi-cancer cohort using the recombinant malaria protein rVAR2

Caroline Løppke[1]
Nicolai T. Sand[2], Kristoffer S. Rohrberg[3], Tobias Gustavsson[1], Thor G. Theander[1], Ali Salanti[1] and Mette Ø. Agerbæk[4]
[1] Centre for Medical Parasitology, Department of Immunology and Microbiology, University of Copenhagen, Denmark
[2] VarCT Diagnostics, Copenhagen, Denmark
[3] Department of Oncology, Centre for Cancer and Organ Diseases, Rigshospitalet, Denmark
[4] Centre for Medical Parasitology, Department of Immunology and Microbiology, University of Copenhagen, Denmark / VarCT Diagnostics, Copenhagen, Denmark

Background & objectives

Isolation of circulating tumor cells (CTCs) from patient samples is clinically valuable as it allows monitoring of real-time disease progression and molecular characterization. A significant barrier towards detection of CTCs is the paucity of tumor-derived cells in the blood and the lack of specific markers to identify these rare cells. Most CTC platforms use epithelial markers for enrichment and validation, which might confound the degree of CTC heterogeneity and prevent detection of biologically relevant subpopulations. We have previously shown that the malaria protein rVAR2 binds specifically to oncofetal chondroitin sulfate chains of proteoglycans on the surface of cancer cells independent of origin or differentiation state. Furthermore, rVAR2 efficiently captures CTCs from pancreatic, prostate, glioblastoma and hepatic cancer patients, making it a potential pan-cancer marker.

We aimed to develop a rVAR2-based method for CTC detection that can be combined with microfluidic size-based isolation for downstream single cell analysis. Furthermore, we aimed to test the feasibility of the rVAR2 assay by CTC enumeration in a multi-cancer cohort (n=25).

Methods

We used rVAR2 coupled to a fluorescently labelled dextran for CTC detection. After blood collection, the red blood cells were lysed and the sample was incubated with a cocktail of rVAR2 or anti-EpCAM, DAPI and leucocyte-specific markers (CD45/16/CD66b) before being deposited on glass slides. The samples were analyzed by fluorescent microscopy, and putative CTCs were defined as rVAR2+/DAPI+/CD45-/CD16-/CD66b-.

For single-cell analysis, an additional 8 ml of blood was stained with rVAR2 prior to isolation on a Parsortix™ device.

Results

In the feasibility study, 36 % (9/25) of the patients had rVAR2-positive CTCs ranging from 1-100 per 4 ml blood. Conversely, no CTCs were detected with anti-EpCAM (0/25). For downstream analysis, 30 % (3/10) of the patients had rVAR2-positive cells after Parsortix™ enrichment, allowing isolation of single cells. In addition, zero CTCs were detected in healthy donor samples (n=10).

Conclusion

The rVAR2-based detection allows identification of CTCs from patients with multiple cancer types. Compared to anti-EpCAM strategies, rVAR2 targets a larger population of CTCs, and can potentially be used to identify a more heterogeneous subset of tumor-derived cells thereby improving the clinical sensitivity of CTC analysis.