Marker-independent enrichment of circulating tumor cells from leukapheresis by non-tumor cell depletion

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Background & objectives

In the future, liquid biopsies from cancer patients could be an attractive support for cancer diagnosis and monitoring the success of therapy. Moreover, detailed molecular characterization of circulating tumor cells (CTCs) could also provide more insights into tumor biology and the process of metastasis. As the analysis of CTCs from blood samples is hampered by extremely low concentrations (1-10 CTCs per ml whole blood), an isolation is necessary before performing downstream analysis. Different enrichment technologies based on immunoaffinity, biophysical properties, or a combination of both are already developed1. The most prominent enrichment technology is the clinically validated and FDA-approved CELLSEARCH® CTC assay system (Menarini Silicon Biosystems), which is based on positive enrichment of CTCs by their expression of epithelial markers, e.g. EpCAM. However, the expression of the epithelial markers used varies in different cancer types and markers as EpCAM are often downregulated in CTCs due to epithelial-to-mesenchymal transition, which means they cannot be used as universal positive selection markers for all tumor entities and CTCs.

We aim for the enrichment of CTCs from blood samples for downstream applications with a marker-independent isolation of CTCs.

Methods

As a model system, cells from the SK-Br3 tumor cell line were spiked into healthy leukapheresis at a frequency of 100-1000 tumor cells per 1e8 leukapheresis cells. Different MACS-based depletion protocols, combinations of selection markers, and microbead titers were tested. Our final protocol is using CD45, CD16, CD31 and glycophorin A as selection

markers, which in combination with the MultiMACS™ Cell24 Separator Plus enables the enrichment of CTCs from large sample volumes.

Results

We were able to develop a workflow for enrichment of CTCs from samples with a frequency of 0.0001% to a final purity of up to 85% and a recovery of up to 75% in the target fraction. Furthermore, we demonstrated that an erythrocyte lysis step is not required in this workflow.

Conclusion

Taken together, the present workflow is a first step to isolate CTCs independently of their tumor origin and marker expression by efficiently depleting the non-tumor cells from the sample, which can pave the way for high-quality downstream analysis.