Enrichment and molecular profiling of circulating melanoma cells: an optimized microfluidic-based workflow

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Abstract

Background: Circulating melanoma cells (CMCs) are responsible for the hematogenous spread of melanoma that ultimately lead to metastasis. Their low abundance in blood as well as the heterogeneous expression of surface markers represent the main limiting factors for their study. The number of CD146-positive CMCs enriched with the FDA-approved CellSearch platform correlates with progression free survival and overall survival, nevertheless, relying on a single marker for CMC enrichment may hamper the identification of all CMC subsets. The Parsortix system allows a more comprehensive phenotypic characterization of all CMCs by the use of customized antibody panels. Moreover downstream Parsotix run, enriched CMC are viable and suitable for any molecular analysis.

Objective: In this study, we tested the strengths and weaknesses of both platforms, integrating CMC count and enrichment with a protocol for their genetic analysis.

Methods: Tumor cells from 23 spike-in and 4 metastatic melanoma samples were enriched using the CellSearch and the Parsortix systems. A customised melanoma antibody cocktail was developed for the Parsortix system to maximize CMC detection. Finally, a paradigmatic uveal melanoma was used to evaluate the entire workflow, including CMC enrichment and an extensive targeted genetic analysis employing custom NGS, ddPCR and MLPA analyses.

Results: Our customized melanoma antibody cocktail efficiently labelled CMCs and differentiated them from endothelial cells/leukocytes. In spike-in samples CellSearch and Parsortix showed comparable capture rate, while in real-life samples Parsortix outperformed CellSearch. A double enrichment process employing both CellSearch and Parsortix systems succeeded in removing most of the leukocyte contamination, resulting in an almost pure CMC sample, suitable for genetic analysis. A proof-of-concept genetic analysis of CMCs from a metastatic uveal melanoma patient revealed multiple genetic alterations, including homozygous GNAQ p.Q209L, hemizygous BAP1 exon 9 deletion, isochromosome 8, and homozygous CDKN2A deletion.

Conclusion: We optimized an approach to successfully enrich and retrieve viable CMCs from metastatic melanoma patients. Moreover, we provided the proof-of-principle for the feasibility of a marker-agnostic CMC enrichment followed by CMC phenotypic characterization and genetic analysis.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.