Post-Enrichment Melanoma Cell Isolation using the VyCAP

Aaron Beasley1, Lisa Oomens2, Michiel Stevens2, Arjan Tibbe2, Joska Broekmaat2, Elin Gray1

1School of Medical and Health Sciences, Edith Cowan University, Perth, Western Australia.
2VyCAP BV, Enschede, The Netherlands

Introduction

Characterisation of melanoma circulating tumour cells (CTCs) might offer the ability to predict prognosis, track genetic evolution of multiple tumours, and may allow detection of mechanisms that confer resistance to treatment. Herein we determined the suitability and efficiency of combining cell enrichment with the Parsortix or immunomagnetic beads followed by the VyCAP system for isolation or enumeration of melanoma CTCs for molecular characterisation.

Applicability

Example of an Applicable Analysis of CTCs. Low-pass whole genome sequencing (WGS) is a low-cost, robust, and informative method of analysing SCNAS in patients. The ability to isolate cells with a high level of efficiency and reliability will enable more routine CTC genomic analysis. Previously we have shown that CTCs isolated from a metastatic UM patient clearly resembled the primary tumour removed 2 years prior and showed features of poor prognosis. Further investigation could offer CTCs as a surrogate to tissue biopsy (Beasley et al., 2018, JCO-PO).

Methods

One uveal melanoma cell line (MP41) and two cutaneous melanoma cell lines (SKMEL5 and A2058) were used for spiking ranging between 100-300 cells into TransFix® CTC (Cytomark) tubes containing 9 ml of, blood and left overnight. Cells were enriched either using Dynabeads (Life Technologies) conjugated with antibodies targeting the melanoma markers ABCB5, gp100, MCAM, and MCSP, or the Parsortix® (Angle) system. The enriched cells were immunostained using the MEL antibody cocktail (anti-MART1/gp100/S100

Capture of Single Cells Enriched with Immunomagnetic Beads

One commonly used method of isolating CTCs is immunomagnetic isolation. However, Dynabeads may pose a problem due to their average size of 2.8 μm causing blockage of the VyCAP microwell chip. Here we spiked samples with 640 μg or 160 μg of Dynabeads to determine a suitable amount of beads.

Conclusions and Future Directions

VyCAP offers a quick, simple, and efficient method for quantification and single cell isolation of pre-enriched melanoma cells. Improvement to on chip staining protocols may further improve cell detection rates without the need of pre-staining in solution, avoiding cell loss. The VyCAP system easily enables isolation of single cells for WGA and molecular analysis.

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