Single-cell transcriptome data enable the identification of novel potential circulating tumor cell-specific markers in non-small cell lung cancer

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Abstract

Background: In the field of Liquid Biopsy, most research projects focus on ctDNA rather than circulating tumor cells (CTC). Nevertheless, by analyzing CTC, significantly more information can be obtained such as the expression of surface markers. However, detection rates with most methods are very low, particularly for non-small cell lung cancer (NSCLC). Using leukapheresis, we recently obtained an unprecedented dataset of 3,363 whole CTC transcriptomes from 6 stage IV NSCLC patients. This single-cell (sc) RNA atlas enabled us to identify 6 potential CTC-specific markers. Here, we present preliminary results of the validation of pyruvate kinase M1/2 (PKM).

Objective: The aim is to utilize our scRNA atlas for the identification of new CTC detection markers.

Methods: Potential CTC markers were identified by analyzing differentially expressed genes between CTC and hematopoietic cells. Protein expression was analyzed via immune cytochemistry (ICC) in NSCLC cell lines H2228, H1975, H1299, H1573, H460, H1650, and SKLU1 spiked into peripheral blood mononuclear cells. Peripheral blood (PB) samples from stage IV NSCLC patients were enriched using a label-independent microfluidic device. CTC were defined as pan-cytokeratin (CK)+, CD45- cells.

Results: PKM, peroxiredoxin 1, annexin A2, lactate dehydrogenase A and S100 calcium-binding protein A10/A11 were identified as candidate CTC specific markers. For PKM, ICC showed protein expression in at least 5 of 7 NSCLC cell lines tested. Regarding the validation of PKM in patient samples, we identified a total of n=242 CTC (CK+, CD45-) from n=20 stage IV NSCLC patient samples (median 4.5, range 0-51). Of these CTC, 97% (234/242) were PKM+ and 3% (8/242) PKM-. We identified 7 PKM+, CK-, CD45- cells. Some CD45+ cells showed low PKM expression.

Conclusion: Preliminary results in patient samples suggest that few additional CTC can be identified by including PKM in the detection of CTC. The utility of PKM as a CTC detection marker may be limited by its expression in some CD45+ leukocytes. However, the heterogeneity in PKM expression may indicate varying dependencies on glycolysis, which might have an impact on clinical outcome and treatment response.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.