Single cell transcriptome atlas reveals a high degree of hetereogeneity in NSCLC circulating tumor cells

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Background & objectives

Background: Circulating tumor cells (CTCs) hold a tremendous potential for a better representation of intratumor heterogeneity and a better understanding of treatment resistance mechanisms. In non-small cell lung cancer (NSCLC) their application is challenged by the limited number of CTCs that can be detected within standard 7.5ml peripheral blood samples. Diagnostic leukapheresis (DLA) has been used to increase CTC numbers, but methods for enrichment of CTCs from such large blood volumes are missing.

Objectives: We aimed to establish a workflow for enrichment of CTCs from DLA and evaluate its potential for detection of intratumor heterogeneity.

Methods

Mononucleated cells and CTCs were collected via DLA from 6 NSCLC patients (stage IV). $80x10^8$ cells (~ 1/3 of each DLA) were processed in a two-step enrichment including the MACS based negative depletion of cells expressing CD45, CD16, CD3, CD31 and CD235a and FACS sorting for $CD45^-$ cells, followed by scRNAseq using 10X Genomics. CTCs were identified through marker gene expression, expression of lung and cancer associated genes and inferred copy number variation analyses.

Results

By pooling data from 6 DLAs, a total of 3,363 NSCLC CTCs transcriptomes were identified. CTCs showed a high degree of heterogeneity along the EMT axis. Using pseudotime and gene set enrichment analyses (GSEA) distinct phenotypes were identified: (i) epithelial-like/highly proliferative/immune responsive (expression of genes for E-Cadherin, Ki67 and enrichment of INF α/γ-response and TNF-α-response via the NFκB pathway), (ii) mesenchymal/invasive/glycolytic (expression of vimentin, hypoxia, mTORC1 and glycosis pathways) and (iii) mesenchymal/cancer stem cell-like (enrichment of ALDH1, oxidative phosphorylation and adipogenesis pathways).

Comparison with single cell transcriptomes from NSCLC- primary tumors revealed a distinct gene signature for CTCs with an upregulation of genes involved in the hemoglobin pathway and downregulation of genes involved in the production of surfactant, amongst others. GSEA indicated higher enrichment of genes involved in cell cycle, migration and invasion indicating an overall higher malignant potential of CTCs.

Conclusion

Enrichment of CTCs from DLAs led to the transcriptomic analysis of an unprecedented number of NSCLC-CTCs. Together with the high CTC heterogeneity, these data clearly indicate the potential of CTC detection from DLA for a better understanding of CTC biology, tumor heterogeneity and ultimately resistance mechanisms.