Advancing Single-Cell Proteomics with ZeptoCTC: A High-Sensitivity Method for Analysis of Circulating Tumor Cells

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Background & objectives

Proteomic analysis of circulating tumor cells (CTCs) might provide a deeper understanding of cancer than genomic profiling alone, as it highlights changes in protein activity, which may not always result from mutations. Our developed single-cell protein analysis workflow, ZeptoCTC, integrates mature technology modules to evaluate protein expression and activation in the PI3K/AKT/mTOR signaling pathway, addressing the limitations in current single-cell proteomic techniques.

Methods

After various optimizations, including modifications to hardware and software and identification of an optimal lysis buffer, the workflow was optimized to analyze AKT1 expression and phosphorylation first in high AKT1 phosphorylated MCF-7 cells treated with 5 µM Capivasertib. Next, using CTC-positive Diagnostic leukapheresis (DLA) products from two patients with MBC, we aimed to determine the discrimination ability between CTCs with and without the AKT1 (E17K) mutation. Our protocol involved micromanipulating single cells, lysis, and printing onto a ZeptoCHIP™ using CellCelector™, followed by antibody-based reverse-phase protein detection using a ZeptoREADER™ and Image J analysis.

Results

ZeptoCTC showed high reproducibility with a signal difference below 10% between replicates. The optimal conditions were determined as 1:1 lysis buffer mixture, spotted in 2 nL volumes. Single MCF-7 treated with Capivasertib exhibited elevated p-AKT/AKT ratios (186%) compared to untreated control cells. AKT1 (E17K) mutated CTCs from one MBC patient revealed higher pAKT levels and a 200% increase in pAKT/AKT ratios compared to patient-matched WBCs ($p = 0.007$, $p = 0.025$). In CTCs from patient 2 harboring CTCs of AKT1 WT-genotype phosphorylation of AKT was not significantly increased.

Conclusion

By integrating advanced single cell isolation and RPPA-analysis ZeptoCTC is a highly sensitive method to measure the expression and phosphorylation of treatment-relevant proteins in key cancer-driving signaling pathways.