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# Isolation, characterization, and expansion of heterogeneous circulating tumor cell (CTC) populations from cancer patients using microfluidic technologies

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Circulating tumor cells (CTCs) are cancerous cells that shed from a primary tumor, intravasate into blood, travel through the blood circulation, and then extravasate to distant organs and form secondary tumors. Hence, CTCs are critical to understand the biological process of metastasis and could serve as potential blood-based surrogate markers to noninvasively evaluate tumor progression and response to treatment.

Although isolation of CTCs from pancreatic adenocarcinoma (PDAC) patients is feasible, investigating their clinical utility has proven less successful than in other cancers due to limitations of epithelial cellular adhesion molecule (EpCAM)-only based CTC assays. We developed a "Carpet Chip" using sequential immunoaffinity-based microfluidics to study the biological relevance of heterogeneous CTCs. Both epithelial (EpCs) and epithelial-to-mesenchymal transition (EMT)-like CTCs (EMTCs) were detected from the blood of PDAC patients (n=35). Thirty-four patients had ≥5 EpCs $mL^{-1}$ and 35 patients had ≥15 EMTCs $mL^{-1}$. Overall, significantly higher numbers of EMTCs than EpCs were recovered, reflecting the aggressive nature of PDAC. Furthermore, higher numbers of EMTCs were observed in patients with lymph node involvement compared to patients without. Gene expression profiling of CTCs from 17 patients revealed that CXCR1 is significantly upregulated in EpCs, while known stem cell markers POU5F1, or Oct-4 and MYC were upregulated in EMTCs. Thus, successful isolation and genomic profiling of heterogeneous CTC populations were demonstrated, revealing genetic signatures relevant to patient outcomes. Individualizing therapies targeting genes involved in EMT could reduce metastasis and improve patient survival.

In further studies on PDAC patients, the utility of CTCs as a tool was validated for assessing tumor response to the only three therapy options currently available: surgery, chemotherapy, and radiotherapy. For all treatment options, we observed a statistically significant decrease in CTC counts after treatment completion, which was associated with prolonged OS.

Orthogonally, in an effort to develop label-free technologies for CTC isolation, a microfluidic Labyrinth device for high throughput, label-free, size-based isolation of CTCs was applied to study CTCs from metastatic non-small cell lung cancer patients (NSCLC). Current methods for isolation of lung CTCs mostly rely on biomarker dependent antibody-based capture, missing populations that may be stem-like in nature. Using Labyrinth operating at a flow rate of 2.5 mL/min, heterogeneous CTC populations were isolated from NSCLC patients (n=21). Detected populations included CTCs (PanCK+ and CD45-), CTCs expressing EpCAM or Vimentin, and CTCs expressing both markers representing an EMT-like population of CTCs. We were able to isolate CTCs from 100% of patients with an average yield of 180±168 CTCs $mL^{-1}$. Among captured CTCs, EpCAM- CTCs were significantly more common than EpCAM+ CTCs (115.7 vs. 39.1 CTCs $mL^{-1}$ respectively). Cell clusters of 2 or more CTCs were also observed in 95% of patients; 79% of these clusters were negative for EpCAM expression, whereas 35% expressed Vimentin, suggestive of an EMT phenotype. Recovered CTCs from patients with *RET*, *ROS1* and *ALK* rearrangements in tumors showed aberrations matching the primary tumor for each gene using FISH analysis. We successfully expanded CTCs *in vitro* and the cultured cells carried matched mutations. The Labyrinth device demonstrated the advantages of marker-independent separation methods for isolation of heterogonous CTC sub-populations in lung cancer for CTC expansion, allowing drug testing for therapies targeting specific driver mutations.

The capability of collecting recovered CTCs from the device using a continuous processing technique opens up opportunities for not only CTC expansion off-chip, but also ex-vivo drug testing to direct patient-specific therapies.