Rare Events with Large-Impact:
Bioengineering & Clinical Applications of Circulating Tumor Cells

Mehmet Toner, PhD
Helen Andrus Benedict Professor of Biomedical Engineering
Massachusetts General Hospital, Harvard Medical School
Harvard-MIT Health Sciences & Technology

Viable tumor-derived circulating tumor cells (CTCs) have been identified in peripheral blood from cancer patients and are probably the origin of intractable metastatic disease. However, the ability to isolate CTCs has proven to be difficult due to the exceedingly low frequency of CTCs in circulation. We introduced several microfluidic methods to improve the sensitivity of rare event CTC isolation, a strategy that is particularly attractive because it can lead to efficient purification of viable CTCs from unprocessed whole blood. The micropost CTC-Chip ($^{\mu p}$CTC-Chip) relies on laminar flow of blood cells through anti-EpCAM antibody-coated microposts, whereas the herringbone CTC-Chip (^{Hb}CTC-Chip) uses micro-vortices generated by herringbone-shaped grooves to efficiently direct cells toward antibody-coated surfaces. These antigen-dependent CTC isolation approaches led to the development of a third technology, which is tumor marker free (or antigen-independent) sorting of CTCs. We call this integrated microfluidic system the CTC-iChip, based on the inertial focusing strategy, which allows positioning of cells in a near-single file line, such that they can be precisely deflected using minimal magnetic force. We applied these three microfluidic platforms to blood samples obtained from lung, prostate, breast, colon, melanoma, and pancreatic cancer patients. We isolated CTCs from patients with metastatic non-small-cell-lung cancer and identified the EGFR activating mutation in CTCs. We also detected the T790M mutation, which confers drug resistance. We also applied microchip to isolate CTCs from blood specimens of patients with either metastatic or localized prostate cancer, and showed the presence of CTCs in early disease. Remarkably, the low shear design of the ^{HB}CTC-chip revealed micro-clusters of CTCs in a subset of patient samples. Microscopic CTC aggregates may contribute to the hematogenous dissemination of cancer. More recently, we used microfluidic capture of CTCs to measure androgen receptor (AR) signaling readouts before and after therapeutic interventions using single-cell immunofluorescence analysis of CTCs. The results support the relevance of CTCs as dynamic tumor-derived biomarkers, reflecting “real time” effects of cancer drugs on their therapeutic targets, and the potential of CTC signaling analysis to identify the early emergence of resistance to therapy. We also characterized epithelial-to-mesenchymal transition (EMT) in CTCs from breast cancer patients. While a few primary tumor cells simultaneously expressed mesenchymal and epithelial markers, mesenchymal cells were highly enriched in CTCs, and most importantly, serial CTC monitoring suggested an association of mesenchymal CTCs with disease progression suggesting a role for EMT in the blood-borne dissemination of human breast cancer. This presentation will share our integrated strategy to simultaneously advance the engineering and microfluidics of CTC-Chip development, the biology of these rare cells, and the potential clinical applications of circulating tumor cells.