

Catch Me If You Can: Isolating Circulating Tumor Cells from Flowing Blood

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Since the discovery that the concentration of circulating tumor cells (CTCs) in blood is a strong predictor of survival rate in metastatic breast cancer (1), there has been great enthusiasm for the potential of these cells as a marker for cancer progression. The technical challenge is to isolate these rare cells (1–100 cells/mL) from whole blood with sufficient sensitivity and specificity, while maintaining cell viability for postcapture analysis. The most widespread methods of CTC isolation are based on immunocapture of antigens highly expressed on CTCs. Epithelial cell adhesion molecule and prostate-specific membrane antigen are the most extensively characterized antigens used for immunologic detection of carcinomas (2). These antigens are often used in conjunction with cytokeratin, an epithelial marker, to discriminate CTCs from leukocytes.

Immunocapture methods can be divided into bead-based approaches and microfluidic approaches. In bead-based approaches, antibody-coated magnetic nanoparticles bind to CTCs in a suspension of blood cells, and the CTCs are then separated with a magnetic field. This is the basis for Veridex's CellSearch®, the only CTC-enumeration assay cleared by the US Food and Drug Administration (3). The disadvantage of this assay is that it requires multiple processing steps—centrifugation, dilution, capture, separation—and leaves the captured cells nonviable. In microfluidic approaches, whole blood is perfused through channels within which cells collide with antibodies immobilized on the channel walls or obstacles within the channel (4–6). Microfluidic methods are compatible with whole blood, thus minimizing sample preparation, and the captured CTCs remain viable. Throughput is limited, however, to about 1 mL of whole blood per hour. The reason for this low throughput is that the rate of formation of the antigen–antibody bond is relatively slow. Consequently, if blood is perfused at a faster rate, cells will not adhere to immobilized antibodies.

In this issue of *Clinical Chemistry*, Hughes and colleagues describe a CTC-isolation device for overcoming the inherent limitation of immunocapture by mimicking the endogenous cell adhesion process (7). In the vasculature, CTCs escape the fast-moving bloodstream and adhere to the vessel wall by forming fast but weak bonds that allow them to roll and slow down before firmly adhering and transmigrating the endothelium (8). Cell rolling on endothelial cells is mediated by E-selectin, a cell adhesion molecule that is also responsible for recruiting leukocytes as part of the inflammatory response. The authors' approach is to coat a microtube with a mixture of E-selectin and an antibody. In experiments with diluted whole blood spiked with a known concentration of hematopoietic cancer cells, the mixture of selectins and antibodies yielded significantly higher recoveries of CTCs than with antibodies alone. Another notable feature is the use of a simple off-the-shelf tube that is the size (300 μm) and shape of postcapillary venules where endothelial cells are stimulated by inflammatory stimuli. A capture efficiency of 50% was achieved at a flow rate of 4.8 mL/h, which is 5-fold higher than most flow-based immunocapture methods and comparable to the highest throughput reported for a microfluidic device (5).

A drawback of this approach is that the purity is not particularly high, because leukocytes also adhere to selectins. The authors show, however, that altering the topography of the tube wall with adsorbing nanoparticles inhibits leukocyte adhesion and spreading compared with smooth surfaces. This surface modification pushes the captured-cell purity from 37% to 66%. The mechanism for this phenomenon is unclear, but there is some evidence that rough surfaces are less inflammatory than smooth ones (9).

The microtube method compared favorably with the CellSearch assay with a limited data set ($n = 12$) of patients with breast, prostate, lung, and ovarian cancer. CTCs were captured with the microtube method from all 12 cancer patients, whereas the CellSearch assay identified CTCs in only 7 of the samples. In addition, the number of cells captured by the microtube was higher than with the CellSearch assay for every sample. Three of the samples from 8 healthy donors were positively identified for CTCs with the microtube method. This result seems like a high false-positive rate; however, the number of cells captured from these samples

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from healthy individuals was <12, whereas for the cancer patients the number of cells captured was >30 for nanoparticle-coated microtubes. The sample size for this study is too small to determine if this difference between the false positives and the true positives is sufficient to discriminate between the 2 groups. Nevertheless, the simplicity, capture efficiency, and throughput of the microtube method warrant further investigation as a potential clinical assay.

Several questions remain unresolved with regard to the clinical utility of CTC enumeration. Cancer cells, even circulating ones, are a heterogeneous population. Do these isolation techniques select for certain subpopulations? And, are these subpopulations the ones we should be worried about? For example, CTCs undergoing the epithelial–mesenchymal transition downregulate cytokeratin, a common positive-selection marker. Epithelial–mesenchymal transition cells have a particularly aggressive phenotype and are likely to be underrepresented by methods that rely solely on immunocapture (10). Another potential issue is that some cancers do not express common biomarkers, such as epithelial cell adhesion molecule, at all. Finally, enumeration is a first step, but postcapture genotypic and phenotypic profiling may yield more-specific information about cancer progression (11). This consideration leads to the vexing question as to how to capture and release CTCs without changing their phenotypic identity. Little research has been done to measure gene regulation and protein production in response to immunocapture.

Adding additional specificity may require combining measurements of immunologic markers with measurements of other physical properties of CTCs. Two promising candidates are the mechanical and electrical properties of CTCs. Mechanical properties, such as elasticity, are markers of carcinomas and can be probed by tracking deformation in microscale flows (12, 13). Similarly, electrokinetic measurements such as dielectrophoresis can detect changes in membrane capacitance, which is another property that distinguishes CTCs from other blood cells (14). Biochemical, mechanical, and electrical measurements can be achieved in a single device that uses lab-on-a-chip technology. A recent example is a device that combines immunocapture from whole blood with capacitance-based enumeration and electrokinetic manipulation for enrichment (15). With the integration of multiple separation metrics that enhance specificity, it is likely that CTC detection will find a place alongside imag-

ing in the monitoring of tumor progression and metastasis.

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