DEFORMABILITY BASED SEPARATION OF CIRCULATING TUMOR CELLS FROM PATIENTS WITH CASTRATE RESISTANT PROSTATE CANCER

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INTRODUCTION

We present a highly effective method for deformability-based separation of circulating tumor cells (CTCs). CTCs have been implicated as potential seeds of cancer metastasis and have strong prognostic and diagnostic value in cancer therapy. The primary challenge in CTC characterization is their extreme rarity in circulation relative to leukocytes. Conventional strategies employ CTC immunoenrichment that is highly selective but may fail to enrich for CTCs with poor antigen expression. However, CTCs exhibit unique morphological characteristics that distinguish them from leukocytes and deformability-based sorting mechanisms represent a compelling alternative label-free CTC enrichment strategy.

MECHANISM AND METHODS

Our group previously reported the microfluidic ratchet mechanism capable of highly selective deformability based cell separation without clogging. This mechanism uses oscillatory flow through tapered constrictions to enable selective unidirectional transport. Here, we developed a continuous version of this process that obviates the need for microvalves and operate with dramatically increased throughput. Implementation of the microfluidic ratchet consists of a 32x2048 matrix of funnel constrictions with microchannels for flow control (Fig. 1). The openings of the funnel constrictions are gradually reduced from the bottom row to the top row, ranging from 18 μm to 2 μm. Cells enter at the bottom-left of the funnel matrix and are driven by a rightward flow simultaneously as a vertical oscillatory flow. Each cell traverses through the funnel matrix in a step-wise diagonal path until reaching a limiting funnel size, at which point the cell moves horizontally towards the outlet (Fig. 2). CTCs are the least deformable cells and reach their limiting funnel size relatively quickly. Leukocytes are more deformable and travel to a smaller funnel region. Finally, erythrocytes are extremely deformable and exit through the top row. Separated samples are then stained for cytokeratin (CK), EpCAM, CD45, and DAPI and scanned by confocal microscope with analysis of the emission spectrum (Fig. 3, 4).

RESULTS

We evaluated the selectivity of this mechanism using UM-UC13 bladder cancer cells doped into whole blood from healthy donors. UM-UC13 cells were enriched by ~10^4 relative to leukocytes, with ~90% capture efficiency, and thus demonstrate significantly greater selectivity than separation based solely on size. We used the microfluidic ratchet device to enumerate CTCs from 39 samples with 33 patients with castrate resistant prostate cancer, in parallel with CellSearch, and 6 healthy control samples. The microfluidic ratchet device found CTCs (>5) in 25/33 patients with an average count of 235, while CellSearch found CTCs (>5) in 12/33 with an average count of 104. In the 20 patients where CTCs were not found by CellSearch, 13 were found with more than 5 CTCs by microfluidic ratchet. In fact, 5 of these patients had CTC counts from 60 to 1519. These results suggest that deformability based separation may select for distinct populations of CTCs than antigenic selection.

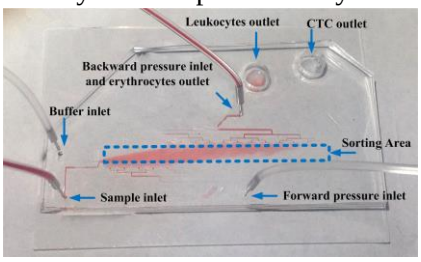

Fig. 1: Photo of the microfluidic ratchet device.

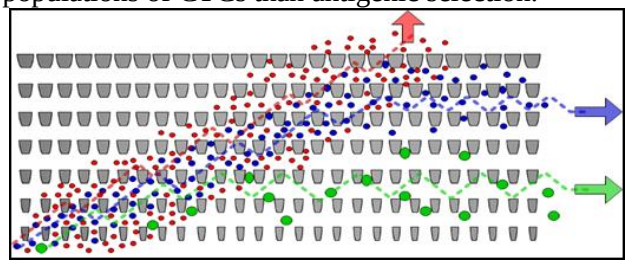

Fig. 2: Operation of the microfluidic ratchet mechanism

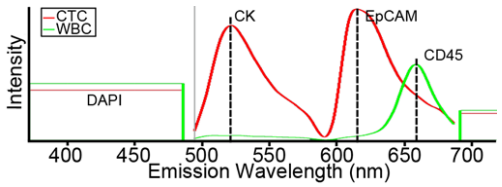

Fig. 3: Spectrum analysis for CTC identification

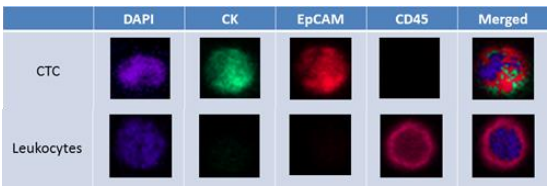

Fig. 4: Fluorescent images of patient CTC and leukocyte.