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Original article

Microfluidic enrichment of circulating tumor cells in patients with clinically localized prostate cancer

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Abstract

Background: Circulating tumor cells (CTC) have become an important tool in the monitoring of patients with advanced prostate cancer (PC). The role of CTC in localized disease has been addressed by only few studies. However, results of CTC analyses are strongly dependent on the platform used for CTC enrichment and detection. In the present study, a microfluidic platform allowing for antigen-independent enrichment of CTC was investigated for its ability to detect CTC in patients with clinically localized PC.

Patients and methods: Blood (2 ml) was collected preoperatively from 50 consecutive patients undergoing radical prostatectomy for clinically localized PC. CTC were enriched using a microfluidic ratchet mechanism allowing separation of CTC from white blood cells based on differences in size and deformability. Enriched cells were stained for immunofluorescence with antibodies targeting pancytokeratin, epithelial cell adhesion molecule, and CD45. In 21 patients, we performed staining for the androgen receptor. CTC counts were correlated with clinical and pathological parameters using the Wilcoxon-Mann-Whitney test for continuous parameters and Chi-square test for categorical parameters.

Results: CTC were detected in 25 (50%) patients. The median number of CTC in CTC-positive patients was 9 CTC/2 ml (range: 1–417). Pancytokeratin positive CTC showed expression of androgen the receptor. We observed no correlation between CTC counts and prostate-specific antigen concentration, tumor stage, lymph node stage, or Gleason grade.

Conclusion: In a representative cohort of patients with clinically localized PC, CTC can be detected in a considerable proportion of patients when using a new microfluidic ratchet mechanism. This encourages further studies assessing the prognostic effect of antigen-independent enriched CTC in patients with PC.

Keywords: Biomarker; Blood; Circulating tumor cells; Minimal residual disease; Prostate cancer; Prostatectomy

1. Introduction

Despite surgical treatment with curative intent, a significant proportion of patients with clinically localized prostate cancer (PC) develop tumor recurrence [1]. Currently, risk stratification is mainly based on clinical parameters and pathologic assessment of the prostatectomy specimen. However, parameters enabling a reliable risk stratification of patients with PC are still lacking.

Circulating tumor cells (CTC) have evolved as an important tool for risk assessment and monitoring of patients with metastatic PC [2,3]. Several studies have also shown that even in patients without distant metastases on preoperative imaging, CTC can be detected in the peripheral blood [4–6]. The significance of these cells has not yet been fully elucidated. Studies in bladder cancer and other malignancies have shown that preoperative detection of CTC is associated with worse outcome [7,8].

The rate of CTC-positive patients is mainly dependent on the technique that is used for enrichment and detection

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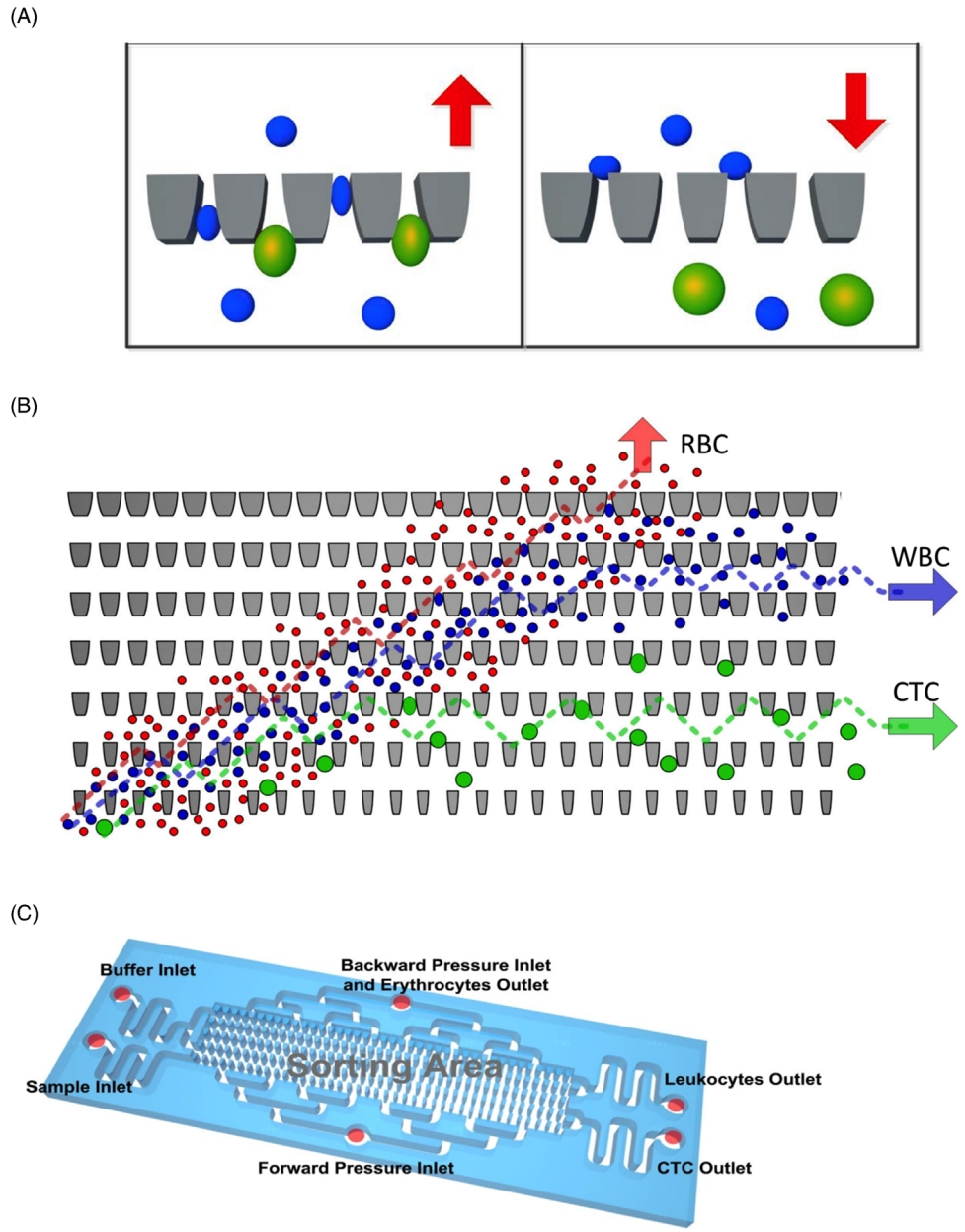

Fig. 1. Microfluidic ratchet mechanism and device operation. (A) Larger and stiff cells are prevented from transmitting through funnel constrictions in forward flow whereas smaller and soft cells are unable to return through funnel constrictions under reverse flow. (B) Device operation. The blood sample infused into the bottom-left of the funnel matrix travels in a diagonal path until reaching a limiting funnel size. The path of the less deformable CTCs (shown in green) flattens at a larger funnel row than the more deformable leukocytes (shown in blue). (C) Design of the microfluidic ratchet device. (Color version of figure is available online.)

of CTC [2,9]. The most broadly available technique, the CellSearch (Janssen Diagnostics, Raritan, NJ) platform, combines immunomagnetic enrichment of epithelial adhesion molecule (EpCAM)-positive cells with immunofluorescent staining for cytokeratins and leukocyte-specific CD45 antigen [10]. However, there is an ongoing discussion that the enrichment based on EpCAM expression might cause failure to detect CTC that have low expression of EpCAM or cells that have undergone epithelial-to-mesenchymal transition, a process that has been closely related to aggressive tumor biology and formation of metastases [11–13]. CTC isolation methods that provide an enrichment based on differences in size using filtration techniques were postulated to overcome this limitation of the CellSearch platform, but these techniques have provided disappointing results for CTC separation because of their lack of selectivity caused by clogging, which occurs when a large number of cells are processed through the filter microstructure [14]. Additionally, separated cells adsorb to the filter, which prevents their extraction for subsequent analysis.

We have developed a microfluidic technology for deformability-based cell separation, which avoids clogging