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Filtration-based enrichment of circulating tumor cells from all prostate cancer risk groups

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Abstract

Objective: To combine circulating tumor cell (CTC) isolation by filtration and immunohistochemistry to investigate the presence of CTCs in low, intermediate, and high-risk prostate cancer (PCa). CTCs isolated from these risk groups stained positive for both cytokeratin and androgen receptors, but negative for CD45.

Patients and methods: Blood samples from 41 biopsy confirmed patients with PCa at different clinical stages such as low, intermediate, and high risk were analyzed. The samples were processed with the ScreenCell filtration device and PCa CTCs were captured for all patients. The isolated CTCs were confirmed PCa CTCs by the presence of androgen receptors and cytokeratins 8, 18, and 19 that occurred in the absence of CD45 positivity. PCa CTC nuclear sizes were measured using the TeloView program.

Results: The filtration-based isolation method used permitted the measurement of the average nuclear size of the captured CTCs. CTCs were identified by immunohistochemistry in low, intermediate, and high-risk groups of patients with PCa.

Conclusion: CTCs may be found in all stages of PCa. These CTCs can be used to determine the level of genomic instability at any stage of PCa; this will, in the future, enable personalized patient management.

Keywords: Prostate cancer; Circulating tumor cells; Androgen receptors; Cytokeratin; Filter isolation

1. Introduction

The description of tumor cells in the blood circulation by Thomas Ashworth [1], ushered a promising field in cancer research where neoplastic cells are isolated then characterized for prognostic and therapeutic design purposes. However, the field of circulating tumor cell (CTC) research has been challenged by the rarity of CTCs in circulation (numbers as low as 1 CTC in every 1 million blood cells have been proposed) [2], the presence of multiple CTC subpopulations in the same patients with cancer [3], the short life span of CTCs in bloodstream and adaptations of CTCs to circulation [4], which leads to loss of unique attributes observed in the parent tumor [3,5,6].

Despite these challenges, many techniques have evolved for the isolation of CTCs based on their physical and immunological properties, with varying results. The size-based filtration technique was chosen for this study owing to its ability to isolate CTCs without the need for antibody detection [3,4,7–9]. The filtration technique preserves the CTC morphology and maintains the presence of CTC clusters. The ScreenCell filters [4], used in this study, are adapted for key biochemical analysis such as FISH, immunohistochemistry, cell culture, PCR, and three-dimensional (3D) imaging [3].

Prostate cancer (PCa) is the most common solid neoplasm in men in western countries with a high incidence rate of 24% of all new cancer cases and 10% of all cancer deaths in

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Canada according to the Canadian Cancer Society [10]. Inaccurate clinical staging of PCa leads to avoidable surgical intervention with associated complications in some patients. Data from CTC research present an opportunity to potentially improve diagnosis, prognosis, and the overall management of PCa. This study documents an effective PCa CTC isolation technique coupled with staining for androgen receptor (AR); cytokeratin 8, 18, and 19; CD45 negativity; and nuclear DNA with 4', 6-diamindino-2 phenylindole (DAPI) to enable subsequent analyses of verified PCa CTCs.

2. Materials and methods

2.1. Patients

Overall, 41 patients with biopsy confirmed PCa were included in this study. Further, 5 low-risk patients, 34 intermediate-risk patients, 3 high risk, and 3 female control blood samples from patients with no medical history of cancer (Table). The patients were risk classified according to the D'Amico classification for survival, which makes use of the clinical staging (TNM), Gleason score of biopsy, prostate specific antigen, and age of the patient [11]. The Research Ethics Board on human studies at University of Manitoba approved the study with ethics reference number HS14085 (H2011: 336). Blood samples analyzed were obtained from patients before surgery. During analysis of the samples, clinical data were blinded to eliminate potential bias.

2.2. Filter device

ScreenCell filtration, a size-based enrichment technique with European CE mark and Health Canada approval (License no. 7044), was used to isolate PCa CTCs from the blood of patients with PCa. It consists of a filter membrane attached to a metal ring placed between a filtration reservoir and a removable nozzle holder (ScreenCell). The filter membrane contains pores with diameter of 7.5 ± 0.36 μm that are randomly distributed throughout the membrane (1×10^5 pores/cm^2). Overall, 4 ml of ScreenCell buffer (ScreenCell) are incubated at room temperature with 3 ml of blood for 8 minutes. The ScreenCell buffer added to the blood sample lyses the red blood cells, prefixes all nucleated cells present in the sample while preserving their architecture and enabling their fixation onto the filter membrane of the device. Thereafter, this mix is passed through the microporous membrane filter, the process of filtration takes less than 5 minutes. Desitter et al. [4] validated this technique by filtration of CTC-spiked cells and this resulted in an average CTC recovery rate of 91.2%. Biochemical processing of the CTCs such as immunohistochemistry, DNA staining, or FISH can be subsequently done on the filters.

3. Immunohistochemistry with cytokeratin and androgen receptor antibodies

3.1. Cytokeratin staining of CTCs

CTCs on the filter are fixed with 3.7% formaldehyde/1× phosphate buffered saline (PBS) for 10 minutes, washed 3 times 3 minutes each with 50 mM $MgCl_2$/1xPBS, and blocked with 50 μl of fetal bovine serum for 30 minutes. The filters are then incubated for 45 minutes at 37°C in a humidified chamber after adding 50 μl of 5 μg/ml cytokeratin 8, 18, and 19 (Abcam Inc., Toronto, Ontario, Canada). The filters are washed, blocked, and 50 μl of Goat antimouse IgG-Alexa 488 (1 μg/ml) (Life Technologies Inc., Burlington, ON, Cat #A11029, Lot/Batch 44094A) are added to incubate for 45 minutes. The filter placed onto microscope slides, covered with cover slips after a coat with Vectashield (Vector Laboratories, Burlington, Ontario, Canada) reagent to minimize photo bleaching.

3.2. Combined androgen receptor and CD45 staining of CTCs

Captured cells on the ScreenCell filters are incubated at room temperature with 3.7% formaldehyde/1× phosphate buffered saline (PBS) for 10 minutes, washed 3 times 3 minutes each with 50 mM $MgCl_2$. Permeabilized with 0.1% Triton X-100 (in ddH_2O) for 12 minutes no shaking, washed 3 times 3 minutes each with 50 mM $MgCl_2$/1xPBS. The filters were incubated for 45 minutes at 37°C in a humidified chamber after 25 μl of AR antibody (441): sc-7305 (Santa Cruz Biotechnologies Inc., Dallas, TX) conjugated with Alexa Fluor 488 at 4 μg/ml and 25 μl of rat antihuman CD45 (Clone YAML501.4; Ref: MA5-17687; Thermo Fisher: 3747 N. Meridian Road Rockford, IL 61105 USA) were applied to the cells on the filter. The filters with the cells go through 3 washes 3 minutes each, and are then mounted on slides. Vectashield (Vector Laboratories, Burlington, ON, Canada) with DAPI was applied on them to stain the nuclear DNA.

4. Three-dimensional image acquisition

Images are acquired using a Zeiss AxioImager Z2 microscope (Carl Zeiss Ltd., Toronto, ON), equipped with AxioCam HR B&W camera and 63×/1.4 oil objective. Thirty 3D interphase nuclei of captured PCa CTCs were imaged and deconvolved for analysis (Fig. 1A). Deconvolution of the images was performed with a constrained iterative algorithm [12]. The reconstructed 3D images were then exported as tif files into TeloView (3D Signatures Inc., Winnipeg, MB, Canada) program for analysis [12].