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Original article

Dynamics of three-dimensional telomere profiles of circulating tumor cells in patients with high-risk prostate cancer who are undergoing androgen deprivation and radiation therapies

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Abstract

Introduction: Accurate assessment and monitoring of the therapeutic efficacy of locally advanced prostate cancer remains a major clinical challenge. Contrary to prostate biopsies, circulating tumor cells (CTCs) are a cellular source repeatedly obtainable by blood sampling and could serve as a surrogate marker for treatment efficacy. In this study, we used size-based filtration to isolate and enumerate CTCs from the blood of 20 patients with high-risk (any one of cT3, Gleason 8–10, or prostate-specific antigen > 20 ng/ml), nonmetastatic, and treatment-naive prostate cancer before and after androgen deprivation therapy (ADT) and radiation therapy (RT).

Materials and methods: We performed 3D telomere-specific quantitative fluorescence in situ hybridization on isolated CTCs to determine 3D telomere profiles for each patient before and throughout the course of both ADT and RT.

Results: Based on the distinct 3D telomere signatures of CTC before treatment, patients were divided into 3 groups. ADT and RT resulted in distinct changes in 3D telomere signatures of CTCs, which were unique for each of the 3 patient groups.

Conclusion: The ability of 3D telomere analysis of CTCs to identify disease heterogeneity among a clinically homogeneous group of patients, which reveals differences in therapeutic responses, provides a new opportunity for better treatment monitoring and management of patients with high-risk prostate cancer.

Keywords: High-risk prostate cancer; Telomeres; Circulating tumor cells; Biomarkers

1. Introduction

Prostate cancer is the most common noncutaneous malignancy in Canadian men with 25,500 men diagnosed annually (Canadian Cancer Society's Steering Committee 2010). The vast majority of patients present with localized prostate cancer that is commonly stratified into low-, intermediate-, and high-risk disease based on clinical characteristics [1,2]. Treatment options for high-risk patients include radical

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prostatectomy or combined androgen deprivation therapy (ADT) and radical radiotherapy (RT).

Because of the widely varying prognoses among patients within the high-risk classification, there is an urgent need for new, more accurate, and noninvasive prostate cancer biomarkers [3]. Currently, the serum prostate-specific antigen (PSA) is the most frequently used biomarker in the diagnosis and treatment of prostate cancer. However, there is no reliable method of differentiating PSA produced by cancer vs. normal prostate tissue. As a result, PSA monitoring in patients after RT has the potential for inaccuracies because the prostate gland is still intact and continues to produce PSA [4]. In addition, many patients thought to have disease recurrence based on rising PSA levels after radiotherapy do not harbor any disease at all after longer follow-up [5]. However, despite the inaccuracies of PSA to reflect the status of the prostate cancer [6,7], it remains an important parameter in oncological decision-making [1].

Ever since Thomas Ashworth [8] first observed blood circulating tumor cells (CTCs) in 1869, there has been speculation that these cells may provide useful information on the primary tumor mass. CTCs have been identified as a new emerging biomarker for prostate cancer and may provide a wealth of biomarker data to aid in prognosis and disease monitoring of patients before and after treatment [9–12]. Few studies have been published examining the response of CTC after treatment with RT in patients with localized prostate cancer [13].

Using Food and Drug Administration–approved CellSearch as a primary method of the isolation of CTCs, enumeration of CTCs in patient blood has been proposed as a potential prognostic biomarker [14,15]. Although the CellSearch is simple and quick, this antigen-based procedure may preselect for certain CTC subpopulations. The targeted epithelial surface marker EpCAM may not be expressed in sufficient quantities in some CTCs, and those CTCs are inevitably missed [16].

In this study, we have used a filter device (ScreenCell) for size-based isolation of CTCs [17]. This method has been shown to isolate 91.2% of cells in spiked samples while allowing passage of 99.9% of common blood cells [17], and permitting a morphology-based identification of CTCs [18].

A total of 3 ml of blood was collected from the patients with high-risk prostate cancer before treatment (+0m), after 2 months of ADT (+2m), and 2 months after the final fraction of RT (+6m). We enumerated CTCs in the blood of these patients and performed 3D telomere-specific telomere fluorescence in situ hybridization on isolated CTC. Recent research [19,20] has shown in prostate cancer cell lines that the androgen receptor is necessary for the proper functioning of telomeres. Meeker et al. [21] have showed that telomere shortening is an early event in prostate tumorigenesis. Similarly, we have previously shown that 3D organization of telomeres is altered in cancer cells and can serve as an early prognostic tumor marker [22–27]. Here, we provide the first evidence that 4 key parameters of 3D telomere architecture and early indicators of genomic instability (nuclear telomere distribution, presence/absence of telomere aggregates, telomere numbers per nucleus, and telomere size) allow us to stratify our patients with high-risk prostate cancer into 3 distinct groups based on the early dynamics of CTC 3D telomere analysis during ADT [23,28–30,22].

2. Materials and methods

2.1. Patients and isolation of CTCs

A total of 20 patients diagnosed with localized high-risk prostate cancer enrolled in the study. Tumors were considered nonmetastatic in patients with negative results in bone scan (Tc-99m-methylene diphosphonate [MDP]) and computed tomography scan of the chest, abdomen, and pelvis. High-risk prostate cancer was defined as having either cT3, Gleason score 8 to 10, or PSA $>$ 20 ng/ml [1]). This study was approved by the University ethics committee (University of Manitoba Ethics Protocol Reference no. H2011:336). Blood (9 ml) was collected in Vacutainer blood collection tubes (with EDTA as anticoagulant) and processed within 2 hours. Patient blood was processed using the ScreenCell filter method for the separation of prostate CTC [17]. Briefly, patient blood (3 ml) was precleared for 8 minutes with 4 ml of red blood cells' lysis buffer and remaining cells were prefixed before ScreenCell (7.50 $\pm$ 0.36 μm pore size) filtration. Filters with captured cells were stored at 4°C for up to 3 months until used. As a control, blood from 3 healthy female volunteers was processed through the ScreenCell apparatus (Supplementary Fig. S2).

2.2. Enumeration of prostate CTC

Filters were imaged on a Zeiss AxioImager Z2 microscope with a Plan-APOCHROMAT ×40 objective (Zeiss, Oberkochen, Germany). Images were acquired using GenASIs software (ASI, Vista, CA, USA) and then exported using the Tagged Image File (tif) Format. To calibrate parameters used in identifying CTCs, sample images were manually processed by 2 observers. Prostate, prostate CTCs on top of the filter were distinguished from remaining few lymphocytes and other main blood cells captured according to cell shape and nuclear size [31] and features (nuclear size, circularity, and solidity) common to CTCs, were extracted. Using a python algorithm, the tif images were converted into binary images and segmented with a watershed algorithm. CTCs were identified by features they shared with the test group.

2.3. Androgen receptor immunocytochemistry

Filters containing the trapped cells were fixed for 10 minutes in 3.7% formaldehyde/1X PBS (Sigma, Oakville,