Original Study

Detection and Phenotyping of Circulating Tumor Cells in High-Risk Localized Prostate Cancer

Sumanta K. Pal,[1] Miaoling He,[2] Timothy Wilson,[3] Xueli Liu,[4] Keqiang Zhang,[2] Courtney Carmichael,[1] Alejandra Torres,[5] Sonya Hernandez,[5] Clayton Lau,[3] Neeraj Agarwal,[6] Mark Kawachi,[3] Yun Yen,[2] Jeremy O. Jones[2]

Abstract

Circulating tumor cells (CTCs) have established prognostic value in the setting of metastatic castration-resistant prostate cancer. However, their utility in the setting of localized prostate cancer is largely unknown. In the current study, a novel method was used to quantify and characterize CTCs in patients with high-risk localized prostate cancer (HRLPC).

Background: In this study, we aimed to determine the feasibility of identifying CTCs in patients with HRLPC, using a modified isolation procedure using the CellSearch (Veridex) platform, and to assess the expression of stem cell and epithelial-mesenchymal transition (EMT) markers on the CTCs. **Patients and Methods:** Thirty-five patients with HRLPC who had chosen prostatectomy for definitive management were prospectively identified. After obtaining consent, four 30-mL blood draws were performed, 2 before surgery and 2 after surgery. The CTC-containing fraction was Ficoll-purified and transferred to a CellSave (Veridex) tube containing dilution buffer before standard enumeration using the CellSearch system. Loss of E-cadherin expression, a marker of EMT, and CD133, a putative prostate cancer stem cell marker, were characterized using the open channel of the CellSearch platform. CTC fragments were also enumerated. **Results:** Using the modified methodology, CTCs were detectable in 49% of patients before surgery. Although no correlation between CTC count and biochemical recurrence (BR) was observed, the percentages of CD133 and E-cadherin–positive CTC fragments were associated with BR at 1 year. **Conclusion:** Our results suggest that further research into the development of CTCs as prognostic biomarkers in HRLPC is warranted.

Clinical Genitourinary Cancer, Vol. 13, No. 2, 130-6
Keywords: CD133, CellSearch, EMT, Fragment, Stem cell

Introduction

An estimated 15% to 25% of patients with localized prostate cancer develop biochemical recurrence (BR) after definitive intervention with radical prostatectomy.[1,2] This finding suggests the presence of occult disease that exists beyond the boundaries of the prostate. Several strategies have been undertaken to detect this disease. Morgan et al evaluated bone marrow aspirates in a series of 569 patients with localized prostate cancer who were treated with prostatectomy.[3] They found that disseminated tumor cells (DTCs), assessed using flow cytometric methods, were present in most patients (72%) before prostatectomy, and the presence of DTCs served as an independent predictor of BR. Several other studies have similarly reported the prognostic value of DTCs, but the physical burden of a bone marrow biopsy represents a practical challenge that limits the utility of DTCs as a biomarker.[4] Other techniques, including reverse transcription polymerase chain reaction- and flow cytometry-based approaches have also been used to detect disseminated disease in patients diagnosed with organ-confined disease; however, none of these approaches have yet been adopted clinically for a variety of reasons.[5]

Circulating tumor cells (CTCs) are a noninvasive alternative to DTCs. The ease of acquisition of CTCs allows for serial collection

Sumanta K. Pal and Miaoling He contributed equally to this work.

[1]Department of Medical Oncology and Experimental Therapeutics, City of Hope Comprehensive Cancer Center, Duarte, CA
[2]Department of Molecular Pharmacology, City of Hope Comprehensive Cancer Center, Duarte, CA
[3]Department of Urology, City of Hope Comprehensive Cancer Center, Duarte, CA
[4]Department of Biostatistics, City of Hope Comprehensive Cancer Center, Duarte, CA
[5]Clinical Trials Office, City of Hope Comprehensive Cancer Center, Duarte, CA
[6]Department of Medical Oncology, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT

Submitted: Jun 17, 2014; Revised: Aug 5, 2014; Accepted: Aug 25, 2014; Epub: Sep 22, 2014

Address for correspondence: Jeremy O. Jones, PhD, Beckman Research Institute, City of Hope Comprehensive Cancer Center, 1500 E Duarte Rd, Duarte, CA 91010
Fax: 626-301-8233; e-mail contact: jjones@coh.org

1558-7673/$ - see frontmatter
http://dx.doi.org/10.1016/j.clgc.2014.08.014

and analysis, a distinct advantage over DTC acquisition. Furthermore, collection and enumeration of CTCs has been standardized and validated for several clinical conditions.[6] In the setting of metastatic castration-resistant prostate cancer (mCRPC), CTCs can be enumerated through the Food and Drug Administration-approved CellSearch (Veridex) system.[7] Despite the potential predictive and prognostic roles of CTC enumeration in mCRPC, the role of this entity in localized disease has not yet been clearly defined. Here, we confirm that it is feasible to identify CTCs in high-risk localized prostate cancer (HRLPC) patients using the CellSearch platform, as others have shown,[8-10] and that these cells have phenotypes associated with aggressive disease, including expression of markers that suggest stem cell and epithelial-mesenchymal transition (EMT) phenotypes. Expression of stem cell markers on cancer cells has been proposed to define a subset of cells with increased plasticity and a better ability to adapt to selective pressures.[11] CD133 is a cell surface antigen that helps to define a population of prostate and prostate cancer cells with stem cell-like characteristics.[12,13] Therefore, we chose to examine CD133 expression on CTCs as a marker of the stem cell phenotype. EMT, and the reverse of this process, mesenchymal epithelial transition, are considered to be crucial for the progression of many different cancers.[14,15] In prostate cancer, markers of EMT, including loss of E-cadherin expression and overexpression of N-cadherin, correlate with Gleason grade and disease progression after radical prostatectomy, suggesting that EMT is related to more aggressive clinical behavior.[16,17] We reasoned that if we were to detect cells with an intermediate epithelial-mesenchymal phenotype, we should use an early marker of the transition, because the CellSearch platform uses immunocapture of the epithelial cell adhesion molecule, and epithelial cell marker, as a first step in CTC isolation. Therefore, we chose to examine loss of E-cadherin expression on CTCs as a marker of EMT, because this is occurs at the earliest stages of EMT.[18]

Patients and Methods

Patient and Control Sample Selection

Using an institutional review board (IRB)-approved protocol (COH IRB 11020), patients with high-risk, localized prostate cancer (defined according to National Comprehensive Cancer Network criteria (ie, $\geq$ cT3a disease, Gleason score 8-10, and/or prostate-specific antigen [PSA] $>$ 20 ng/mL) who had chosen prostatectomy for their definitive management were prospectively identified. The presence of metastatic disease was ruled out because patients in the current series had evaluations that were negative for metastases using magnetic resonance imaging (MRI) of the abdomen and pelvis before surgery (or computed tomography [CT] scan if contraindications existed), and technetium bone scan. Five volunteers without a known history of cancer were chosen as a negative control population using a separate IRB protocol (COH IRB 12311).

Processing of Blood Samples

Blood was drawn into 4 CellSave (Veridex) tubes and within 2 hours, blood was pooled, 30 mL was measured, and the buffy coat, which contains CTCs, was purified using Ficoll gradient (Figure 1A). The buffy coat was diluted into 7.5 mL of CellSave dilution buffer and transferred to a new CellSave tube, where it was processed according to standard CellSearch procedures using the CTC kit and the Celltracks Autoprep System and the Celltracks Analyzer (both from Veridex). CTCs were enumerated by a trained operator. The blood draw schedule included sample acquisitions 2 weeks before radical prostatectomy (RP), immediately before RP, and at 1 and 3 months after RP (Figure 1B).

Figure 1 **Blood Draw and Experimental Design. (A) At Each Blood Draw, Blood Was Drawn Into 4 CellSave (Veridex) Tubes and Within 4 Hours, Pooled Into 30 mL, Whereupon the Buffy Coat, Which Contains Circulating Tumor Cells (CTCs), Was Purified Using Ficoll Gradient. The Buffy Coat Was Transferred to a New CellSave Tube With CellSave Dilution Buffer to a Total of 7.5 mL, Which Was Processed According to Standard CellSearch (Veridex) Procedures Using the CTC Enumeration Kit. (B) The Blood Draw Schedule Is Shown, Including the Marker That Was Evaluated in the Open Channel of the CellSearch System at Each Draw**