Prostate Cancer Disseminated Tumor Cells are Rarely Detected in the Bone Marrow of Patients with Localized Disease Undergoing Radical Prostatectomy across Multiple Rare Cell Detection Platforms

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Abbreviations and Acronyms

4P = PSA, PAP, PSCA and PSMA cocktail

AR = androgen receptor

BM = bone marrow

CK = pan-cytokeratin

CTC = circulating tumor cell

DTC = disseminated tumor cell

EpCAM = epithelial cell adhesion molecule

FHCRC = Fred Hutchinson Cancer Research Center

GS = Gleason Score

HD-SCA = high definition single cell assay

IC = iliac crest

JHH = Johns Hopkins Hospital

PAP = prostate acid phosphatase

PB = peripheral blood

PCa = prostate cancer

PSA = prostate specific antigen

PSCA = prostate stem cell antigen

PSMA = prostate specific membrane antigen

RP = radical prostatectomy

USC = University of Southern California

UW = University of Wisconsin

VERSA = versatile exclusion based rare sample analysis

Purpose: Prostate circulating tumor cells escape into peripheral blood and enter bone marrow as disseminated tumor cells, representing an early step before conventionally detectable metastasis. It is unclear how frequently this occurs in localized disease and existing detection methods rely on epithelial markers with low specificity and sensitivity. We used multiple methodologies of disseminated tumor cell detection in bone marrow harvested at radical prostatectomy.

Materials and Methods: Bone marrow was harvested from 208 clinically localized cases, 16 controls and 5 metastatic cases with peripheral blood obtained from 37 metastatic cases. Samples were evaluated at 4 centers with 4 distinct platforms using antibody enrichment with the AdnaTest (Qiagen®) or VERSA (versatile exclusion based rare sample analysis), or whole sample interrogation with the RareCyte platform (Seattle, Washington) or HD-SCA (high definition single cell assay) using traditional epithelial markers and prostate specific markers. We investigated the sensitivity and specificity of these markers by evaluating expression levels in control and metastatic cases.

Results: *EpCAM*, *NKX3.1* and *AR* were nonspecifically expressed in controls and in most samples using AdnaTest with no relation to perioperative variables. Only 1 patient with localized disease showed positive results for the prostate specific marker *PSA*. With the VERSA platform no localized case demonstrated

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disseminated tumor cells. With the RareCyte and HD-SCA platforms only a single patient had 1 disseminated tumor cell.

Conclusions: Evaluation across multiple platforms revealed that epithelial markers are nonspecific in bone marrow and, thus, not suitable for disseminated tumor cell detection. Using prostate specific markers disseminated tumor cells were typically not detected in patients with localized prostate cancer.

Key Words: prostatic neoplasms; biomarkers, tumor; neoplastic cells, circulating; bone marrow; neoplasm metastasis

DISSEMINATED tumor cells are cancer cells that escape the primary cancer and enter a target organ, representing a first step toward detectable metastasis.[1] A common landing zone for PCa cells is BM. These cells provide an opportunity for the early detection of cancer spread before the development of clinical metastases that are large enough to be captured on conventional imaging.[2]

Previously Morgan et al reported that DTCs were present in 72% of men with localized PCa prior to RP.[3] Unexpectedly DTCs were not associated with pathological stage, Gleason grade or PSA. Notably this work relied on epithelial markers to detect cancer cells in BM. There has been recent appreciation of the phenotypic diversity of DTCs and the cellular complexity of BM, which has called into question the reliability of traditional epithelial markers such as *CK* and *EpCAM*.[4–8]

The limited sensitivity of epithelial markers was noted recently by McDaniel et al in their study of the detection of CK negative PCa CTCs, which was confirmed by fluorescence in situ hybridization for PTEN and ERG as well as immunofluorescent staining for AR expression.[8] The specificity of epithelial markers is also an issue, given that *EpCAM* is known to be ubiquitously expressed on normal resident cells of BM, including erythroid progenitor cells and human epidermal Langerhans cells.[4,9–11] *AR* is also not an ideal prostate marker in BM as it facilitates the regulation of hematopoiesis with ubiquitous expression in normal male and female BM.[12] Although *NKX3.1* is traditionally considered a prostate specific marker, Uhlén et al found detectable mRNA expression in normal female and male BM tissues.[13]

Given the absence of a proven prostate specific marker that is reliable in the BM niche, little is known regarding when or how often PCa cells disseminate to BM as DTCs. Previous studies have demonstrated that 35% of patients with localized PCa who undergo RP will experience biochemical recurrence within 10 years.[14] Thus, it is imperative to investigate DTCs as an early step in the currently incurable process of metastasis since eventually castrate resistance and disease progression will develop in almost all men with metastatic PCa.[15]

In the current study we used various immunofluorescence and mRNA expression based rare cell detection platforms to evaluate the presence of DTCs in patients with localized PCa using traditional epithelial and prostate specific markers. Additionally, we investigated the sensitivity and specificity of these markers by evaluating expression levels in controls and metastatic PCa cases.

MATERIALS AND METHODS

Disseminated and Circulating Tumor Cell Detection Assays

Two immunofluorescence based assays were used at 2 separate centers, including the HD-SCA at USC and the AccuCyte® at FHCRC (supplementary methods and supplementary Appendix, http://jurology.com/). Additionally, 2 mRNA expression based assays were performed at 2 separate centers, including the AdnaTest (Qiagen) at JHH and the VERSA platform at UW (supplementary methods and supplementary Appendix, http://jurology.com/, and table 1). All methods were verified with spike-in controls. Notably the expression patterns of individual cells cannot be queried by mRNA expression applied to a cell population (fig. 1).

Sample Collection and Distribution

All samples were collected consecutively under institutional review board approved protocols with no reported complications. Samples from JHH were distributed to all centers according to preexisting standards for processing time window and shipping (table 1). Patients were tested on a maximum of 2 platforms, given the harvest volume limitation for BM aspiration. RP BM samples were taken using anesthesia prior to incision. Of BM samples 79% were collected from the pubic bone while 21% were collected from the anterior IC. Of the 106 JHH samples 67 (63%) were pubic bone and 39 (37%) were IC. All 43 USC samples (100%) were pubic bone. Of the 18 UW samples 17 (94%) were pubic bone and 1 (6%) was IC. Of the 41 FHCRC samples 37 (90%) were pubic bone and 4 (10%) were IC.

A total of 18 BM samples from clinically localized cases prior to RP were sent to UW for analysis. Of the 41 localized BM samples sent to FHCRC, those of 2 patients (5%) were also evaluated at UW. A total of 43 localized BM samples with 5 matched PB samples were sent to USC for analysis by the HD-SCA assay. The samples of 16 of these patients (37%) were also evaluated at JHH with the AdnaTest.

At JHH BM samples were collected from 106 patients with clinically localized PCa prior to RP and 16 controls

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