

P3-26: Analyses of circulating tumor cell dynamics and treatment response in prostate cancer using the CTC-Chip microfluidic device

Richard J. Lee*, Shannon L. Stott*, Sunitha Nagrath*, Min Yu, David Miyamoto, Maria Kempner, Lecia Sequist, Douglas M. Dahl, Matthew R. Smith, Mehmet Toner, Shyamala Maheswaran, Daniel A. Haber
Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, MA (*denotes equal contribution)

Abstract

Background: Primary and metastatic cancers shed tumor cells that circulate in the bloodstream. In localized prostate cancer, CTCs may be indicative of tumor invasiveness. In the metastatic setting, CTCs may be useful in monitoring response to therapy.

Objectives:

- To evaluate the utility of the CTC-Chip to quantify CTCs in prostate cancer patients undergoing treatment.
- To perform molecular characterization of captured prostate CTCs.

Results: A semi-automated imaging platform for quantification of PSA-stained CTCs was developed. In localized disease, CTC quantities fell to undetectable levels after surgery, with three general patterns of behavior that may indicate underlying biological differences. In patients with metastatic disease undergoing either ADT or chemotherapy, CTC quantities generally corresponded with changes in serum PSA and radiographic assessments. 45% of tested patients exhibited the *TMPRSS2:ERG* translocation. Ki67, DAPI, PSA, and PSMA staining are feasible. FISH for Androgen Receptor amplification on released nuclei is also feasible.

Conclusions: The highly sensitive CTC-Chip can detect dynamic changes in CTCs in patients undergoing prostatectomy, ADT, or chemotherapy.

Impact: This work has led to larger, ongoing studies evaluating whether patterns of CTC changes predict recurrence or treatment response. A second-generation CTC-Chip has been developed. Molecular characterization of CTCs may provide new insights into prostate cancer biology, clinical behavior, and therapeutic targets.

Background

Prostate cancer accounts for 28,000 deaths per year in the U.S. Prostate cancer metastases in bone are not readily biopsied; molecular insights into metastatic disease are limited.

In localized disease, 1/3 of treated patients will develop a recurrence. Currently, biomarkers do not exist that can reliably distinguish indolent from aggressive disease.

CTCs are a potential source of cells derived from primary or metastatic sites that may be amenable to analysis. The CTC-Chip was developed at our institution and captured CTCs from the majority of solid tumor patients in initial studies. The CTC-Chip is a microfluidic device capable of enumerating CTCs with high yield and purity. Molecular analyses including immunohistochemistry and mutation genotyping have been shown to be possible with the CTC-Chip.

Methods

CTC collection. Patients were recruited and consented according to an IRB-approved protocol. Peripheral blood (20 mL) was collected for CTC-Chip analysis, during routine visits. Treatments (surgery, androgen deprivation therapy, and chemotherapy) were administered as per standard clinical care.

CTC isolation and staining. Peripheral blood is processed within 6h of collection. Whole blood was applied to the CTC-Chip at 1-2mL/h. EpCAM-expressing cells bind to the 78,000 microposts coated with anti-EpCAM antibody. The chip was washed with saline to remove non-specifically bound cells. CTCs were fixed and stained with antibodies for cytokeratin7/8, prostate specific antigen (PSA), DAPI, or Ki67.

Automated imaging and enumeration. An automated digital analysis system was designed to detect PSA(+), DAPI(+), EpCAM-bound cells (see references). Quantities of CTCs were serially monitored with different therapies.

TMPRSS2:ERG analysis. RNA extraction from CTCs or the corresponding prostatectomy specimen of the same patient was performed using an RNA isolation kit. cDNA was generated and subjected to 2 rounds of linear amplification. Nested PCR for the *TMPRSS2:ERG* message was then performed. For FISH analysis of prostatectomy specimens, probes for *ERG* and *TMPRSS2* were hybridized according to standard protocol.

Results

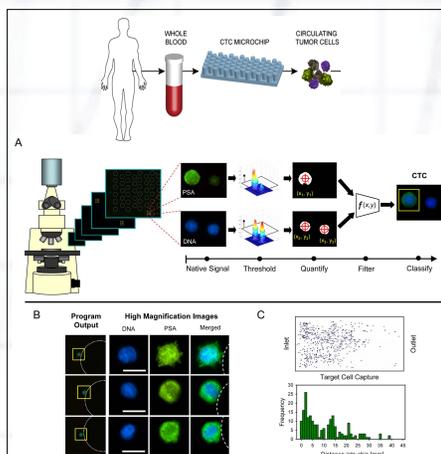


Fig. 1. Capture of CTCs and PSA-based enumeration.
a. Semi-automated detection system. The entire CTC-Chip is imaged in three dimensions using an automated microscopic platform. Post-acquisition, all images are analyzed via an image processing algorithm, with a fluorescence intensity threshold for PSA and DAPI signals. Binarized signals are quantified and sorted; those meeting preset criteria are filtered to ensure that separate DNA and PSA signals are precisely colocalized.
b. Representative images of PSA-positive CTCs from 3 patients. Scale bar = 10 μ m. Using the automated system, 26/50 (52%) prostate cancer patients had detectable CTCs, including 18/31 (58%) with metastatic disease and 8/19 (42%) with localized disease.
c. Representative "heatmap" and the corresponding decay profile of an entire CTC-Chip.

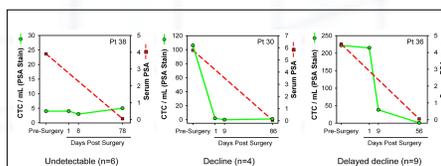


Fig. 2. Serial monitoring of CTCs in localized prostate cancer following radical prostatectomy by the same surgeon. Three subsets of cases were observed, as illustrated. The presence or absence of CTCs or the pattern of resolution was not associated with pre-operative serum PSA level, Gleason score, size of primary tumor, extracapsular extension, lymphatic or perineural invasion, or margin status.

Results

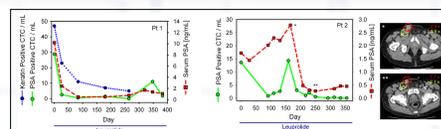


Fig. 3. Serial monitoring of CTCs in patients with metastatic disease.
Left: Treatment with androgen deprivation therapy (ADT), then addition of bicalutamide. Right: ADT, then docetaxel chemotherapy.

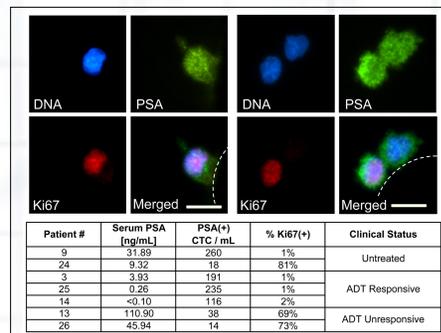


Fig. 4. Proliferative index of PSA-positive prostate CTCs.
Upper panels: CTCs isolated from two patients with metastatic prostate cancer, co-stained for PSA and Ki67. Representative high-resolution images with markers as indicated. Scale bars represent 10 μ m.
Lower panel: Numerical analysis of 7 patients with metastatic prostate cancer at various states of treatment. The percent Ki67 column reflects the fraction of PSA-positive CTCs that are also positive for Ki67 (i.e., the proliferative fraction).

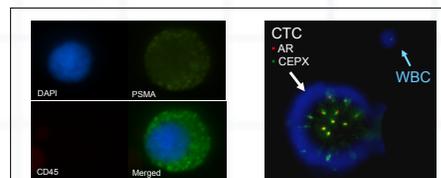


Fig. 5. Other markers: PSMA staining and AR FISH in CTCs.
Left: CTC isolated from a patient with metastatic prostate cancer, co-stained for DAPI, PSMA, and CD45. Representative high-resolution images including merged views.
Right: CTCs from the same patient were captured on the CTC-Chip. Nuclei were released and subjected to fluorescent *in situ* hybridization (FISH) for the androgen receptor (AR). At least 7 copies of AR were identified in this cell.

Results

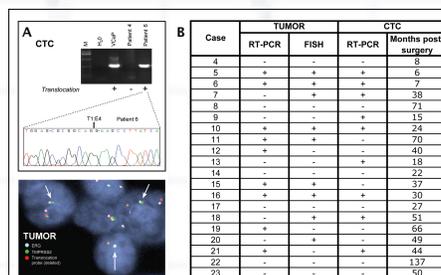


Fig. 6. Detection of *TMPRSS2:ERG* translocation in prostate cancer.
a. cDNAs from CTCs were analyzed by PCR. Two patients, (+) and (-) controls are shown. Sequencing confirmed the translocation. FISH of the same patient's primary tumor.
b. Evaluation of *TMPRSS2:ERG* status in metastatic CTCs vs. primary tumor in 20 cases. Primary tumors were tested by both FISH and RT-PCR, and were concordant for 70% of cases. Concordance between primary tumor and metastatic CTCs was 60-70%.

Conclusions / future directions

PSA staining and automated detection are feasible for prostate CTC analysis. This sensitive and quantifiable technological platform permits noninvasive molecular characterization of prostate CTCs in both localized and metastatic cases.

For localized disease, differences in CTC half-life may reflect important biological properties with clinical implications. Larger, long-term studies are warranted. The Physician Research Training Award from the DOD is supporting Dr. Lee to pursue an ongoing 200 patient study (also supported by a Prostate Cancer Foundation Challenge Award (PI, Daniel Haber)).

CTC quantities and serum PSA did not correlate well across different patients, but showed a close correlation following therapeutic interventions in individual patients.

Molecular characterization of prostate CTCs may yield new biological insights, may distinguish molecularly defined populations, and may identify potential therapeutic targets. The *TMPRSS2:ERG* analyses indicate that a dominant tumor population may evolve during the metastatic process, and highlights the importance of defining tumor genotypes at the time of clinical presentation and therapeutic intervention. The second-generation Herringbone-Chip allows for larger scale production of the Chip with similar or better capture efficiency, and will be used for future studies.

References and funding

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