Abstract

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 menopausal disease are limited. In localized disease, 1/3 of treated patients will develop a recurrence. Currently, Prostate cancer metastases predict treatment response. A second-generation CTC-Chip has been developed. Molecular characterization of CTCs may provide new tools into prostate disease diagnosis, biomarker discovery, and therapeutic approaches.

Objective: To perform molecular characterization of captured prostate CTCs.

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 clinical visits. Treatments (surgery, androgen deprivation therapy, and chemotherapy) were administered after blood collection. CTC isolation and staining: Peripheral blood was processed within 48h of collection. Whole blood was applied to the CTC-Chip at 1-2mL/h. EpCAM-expressing cells bind to the Chip and are captured. CTCs are a potential source of cells derived from primary or metastatic sites that may be analyzed for molecular characteristics. 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.

CTC enumeration: The entire CTC-Chip is imaged in multiple dimensions using an automated digital analysis system. 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. 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. Semi-automated detection system: The Chip was hybridized according to standard protocol. 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. Semi-automated detection system: The Chip was hybridized according to standard protocol. Semi-automated detection system: The Chip was hybridized according to standard protocol.

Results

CTC isolation and staining: Peripheral blood was processed within 48h of collection. Whole blood was applied to the CTC-Chip at 1-2mL/h. EpCAM-expressing cells bind to the Chip and are captured. CTCs are a potential source of cells derived from primary or metastatic sites that may be analyzed for molecular characteristics. 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. A representative “heatmap” of the entire CTC-Chip is illustrated. The presence or absence of CTCs or the pattern of resolution was not illustrated. The percent Ki67 column reflects the fraction of PSA-positive CTCs that are also positive for Ki67 (i.e., the proliferative fraction).

Conclusions & future directions

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 TP53/ERG ESZ analysis indicates 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 Harper-CopiaChip 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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