Molecular Analysis of CTCs in Prostate Cancer

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Circulating tumor cells (CTCs) are rare cancer cells shed from primary and metastatic tumor deposits into the peripheral blood of patients with cancer en route to forming (additional) metastasis. Isolation of CTCs represents a form of “liquid biopsy” that can be performed noninvasively and allows molecular analyses of tumor samples before and after therapy, enabling monitoring of treatment response and yielding clues to resistance mechanisms. This concept is especially applicable to metastatic prostate cancer, in which characteristic bone metastases are often difficult to biopsy.

Because of their rarity and fragility, CTCs have been difficult to isolate and study. In patients with solid tumors, approximately one in one billion blood cells is a CTC. Researchers led by Daniel A. Haber, MD, PhD, and Mehmet Toner, PhD, have made recent efforts to isolate and molecularly characterize CTCs from patients with prostate cancer using highly sensitive microfluidic technologies. The CTC-iChip is a microchip-based device that processes whole blood samples in 3 stages of microfluidic depletion: removal of all blood components except for CTCs and white blood cells (WBCs) based on size; aligning the cells in a single file to allow for precise and rapid sorting; and sorting out magnetically labeled target cells, either CTCs tagged via an epithelial marker or WBCs tagged by antigens (e.g., anti-CD45). Tagging the WBCs leaves behind an enriched population of unlabeled CTCs without relying on the presence of an epithelial marker or other known tumor antigens.

In the clinical setting, CTC analysis has the potential to guide therapeutic decision-making and provide insight into mechanisms of treatment resistance. Agents targeting the androgen receptor (AR), including abiraterone and enzalutamide, are among the most recently approved agents in metastatic castrate-resistant prostate cancer (mCRPC). Several techniques have been proposed to measure changes in AR signaling in CTCs in response to AR-targeting therapies. At the single-cell level, RNA profiling of CTCs reveals intercellular heterogeneity and potential mechanisms of therapeutic resistance.

The development of a quantitative immunofluorescence assay based on the expression of AR regulated genes, rather than independent mechanisms of AR reactivation (e.g., AR amplification or mutation, AR ligand overexpression, or AR cofactor misregulation), enabled the measurement of AR pathway reactivation during the acquisition of resistance to androgen deprivation therapy (ADT). Prostate specific antigen (PSA) is consistently upregulated following AR activation, whereas prostate-specific membrane antigen (PSMA) is consistently upregulated following AR suppression. Therefore, PSA and PSMA serve as reliable biomarkers of AR activation and suppression, respectively. In castration-sensitive patients with newly diagnosed mCRPC who were starting ADT, pre-treatment CTCs demonstrate an “AR-on” phenotype. However, 4 weeks following the initiation of ADT, a population of CTCs demonstrating the “AR-off” phenotype emerges. By comparison, castrate-resistant prostate cancer cells demonstrate a striking heterogeneity of “AR-on,” “AR-mixed,” and “AR-off” CTC phenotypes.

Based on these findings, the research team hypothesized that RNA-sequencing of single prostate CTCs may uncover cellular pathways that underlie disease progression and resistance to therapy. Furthermore, single-cell analyses may enable the study of the molecular and cellular heterogeneity of prostate cancer. One recent study examined the single-cell RNA sequencing profiles of CTCs obtained from 22 patients with prostate cancer using the CTC-iChip device. Of 221 single CTCs isolated, 133 (60%) were successfully amplified and sequenced, and prostate cancer lineage was confirmed in 77 CTCs. RNA sequencing showed considerable heterogeneity in marker expression (e.g., prostate, epithelial, mesenchymal, stem cell, and proliferation markers) in prostate CTCs within individual patients, between patients, and in comparison with cells derived from primary tumors and prostate cancer cell lines. Compared with cells from primary tumors, 21 signaling and regulatory pathways were enriched in prostate CTCs.
Focusing on potential mechanisms of resistance to ADT, the noncanonical Wnt signaling pathway was significantly enriched in CTCs derived from enzalutamide-resistant patients compared to those from enzalutamide-naive patients (p=0.0064). Furthermore, enzalutamide acutely induced the endogenous Wnt-5A ligand in LNCaP cells in vitro, whereas its knockdown suppressed enzalutamide resistance. These findings suggest that noncanonical Wnt-5A plays a key role in the development of resistance to enzalutamide. Multiple AR alternative splice variants were also detected (e.g., AR-V7), even within individual CTCs. Heterogeneous signaling pathways may contribute to antiandrogen resistance in CRPC.

**Summary**

The molecular analysis of CTCs may lead to the identification of drug resistance mechanisms and the discovery of novel non-invasive biomarkers of treatment response and resistance. Although these findings require further clinical validation, they highlight the potential of CTC analyses to help guide the clinical management of prostate cancer patients.

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**References**


