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Circulating Tumor Cells in Prostate Cancer: The Premise and The Promise

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Why Study Circulating Tumor Cells in Prostate Cancer?

Both primary and metastatic tumors shed cells into the bloodstream that circulate, giving rise to metastases that eventually form in other organs. The number of these cells is thought to be only one in one billion blood cells, and because of this, they are technically difficult to isolate and identify. Being able to capture and study circulating tumor cells (CTCs) may provide a noninvasive method to sample the cancer and perform molecular characterization to identify ways to monitor response to treatment. For patients with localized disease, CTCs may provide information about predictions of response to therapies, markers of invasion, and potential methods to detect and diagnose cancer earlier. CTCs may also provide information surrounding the properties of rare metastatic precursors to help understand how metastases occur and to target novel drugs and markers of response to better treat these metastases. While serum prostate specific antigen (PSA) remains a valuable marker, it does not tell the entire prostate cancer (PCa) story. Most prostate tumors metastasize to the bony skeleton, which is very difficult to sample. In addition, recurrence of prostate cancer may occur years after initial therapy, and the molecular changes seen in primary tissue may not actually reflect the biology of recurrent disease. CTCs and the molecular alterations identified within them may provide a different and more accurate biomarker for patients with prostate cancer.

Methods of Studying CTCs

Multiple technologies for CTC detection are being used or are undergoing investigation. Most studies of CTCs have used immunomagnetic sorting with bead-based capture. Other methods include microfluidics, filtering (isolation by size of epithelial tumor cells[ISET[), density-graded centrifugation, flow cytometry, immuno-microbubbles, and epithelial immunospot (EPISPOT). Immunomagnetic capture is the method use by most laboratories, commercially available as a device called CellSearch (Janssen Diagnostics, Raritan, NJ). This CTC isolation method works via positive selection of CTCs expressing an epithelial cell-surface marker (EpCAM). Anti-EpCAM antibody-coated magnetic beads pull out and enrich CTCs from other components with application of a magnetic field, purifying them away from other blood components. However, this technique has limitations, including the fact that cells are fixed prior to staining, preventing other molecular analyses. In addition, its sensitivity is limited by the EpCAM-based detection method, it precludes the ability to use other technologies, and yields tend to be low. CellSearch is currently the only CTC enrichment test cleared for use by the United States Food and Drug Administration (FDA). This approval resulted from a study of 276 patients with metastatic castration-resistant prostate cancer (mCRPC) starting a new line of chemotherapy. Patients were stratified into cohorts having <5 CTCs per 7.5 mL tube of blood versus those with ≥5 CTCs per tube. Investigators demonstrated that patients with fewer (<5) CTCs at baseline lived significantly longer than those with an unfavorable level of CTCs (≥5). CTCs were more significant in predicting outcomes than PSA decline from these treatments [1].

In another clinical study of 164 men with prostate cancer starting first-line chemotherapy, a higher baseline number of CTCs correlated with higher risk of death, suggesting that CTCs may be a useful prognostic biomarker. Interestingly, lactate dehydrogenase (LDH), a common laboratory test, was found to be more prognostic than CTCs, a finding which bears further study [2]. Another study assessed patients with mCRPC starting a trial of abiraterone following docetaxel chemotherapy and followed multiple markers, including CTC count and LDH. While treatment with abiraterone improved survival, high baseline LDH and CTC counts predicted worse survival [3]. These studies have suggested that CTCs at baseline or a change in category (favorable to unfavorable or vice versa) have prognostic value for survival. In addition, CTCs may

have value as a surrogate endpoint for clinical trials, although baseline LDH evaluation appears to have a stronger association with outcome and is less costly and more accessible [2,3]. Many other CTC analyses are ongoing for biomarker development. Laboratories are using the current CellSearch platform to enrich for CTCs and then customizing analyses as needed. Overall, there is room for improvement in this technology.

Microfluidic Capture and Characterization of CTCs

Another technique that has been developed at Massachusetts General Hospital involves use of microfluidic capture for characterization of CTCs. The first-generation technology used a micropost CTC chip with multiple microposts affixed to a glass slide and coated with EpCAM antibodies that would bind CTCs similar to the CellSearch platform. Cells that do not express EpCAM, such as blood cells, would not become bound to the microposts. The bound cells could then be washed and undergo molecular characterization. When tested in patients with prostate cancer, only prostate CTCs were found to express PSA. PSA-positive epithelial cells were bound to the platform as they expressed EpCAM. Ribonucleic acid (RNA) could be isolated from these cells to compare prostate cancer cells with other types of malignancies. Polymerase chain reaction (PCR) amplification demonstrates that the CTCs express PSA at the RNA level [4]. Automated image analysis was also developed to identify PSA-expressing CTCs[5].

In mCRPC, this technology can delineate that a patient's clinical response to therapy correlates with CTC changes. CTCs have been shown to decline with therapy along with a decline of expression of PSA; therefore, CTC changes correlate with PSA responses. The presence of a specific translocation also assisted in confirming that these were prostate cancer cells. About 50% of prostate cancer cells have a fusion event that fuses the TMPRSS2 gene to the ERG gene. The presence of the TMPRSS2:ERG translocation in CTCs confirmed their origin from malignant prostate cancer. However, a concordance of only 70% was found between the CTCs and the primary tumors, suggesting either metastatic evolution or heterogeneity from the primary tumor to the CTCs [5]. For patients with metastatic disease, changes in CTCs may reflect responses to therapy although different tumor populations may evolve over time. It may be possible to define tumor genotypes at the time of presentation, increasing the possibility of more accurate therapeutic intervention. Studies have also been performed in patients with localized disease post-prostatectomy, with patients falling into one of three groups, either those with undetectable CTCs at any time, those with detectable CTCs pre-prostatectomy who exhibited a rapid decline of CTCs by day 1, or those whose CTCs persisted for a week or longer. Data suggested that in patients with localized disease, neither preoperative CTC presence nor pattern of postoperative CTC resolution correlated with PSA, tumor burden, or tumor pathology. Rapid clearance for some patients may reflect the removal of the sole source of CTCs whereas delayed removal may reflect shedding of CTCs from extra-prostatic disease sites. Different patterns of CTC changes after surgery may identify those at higher risk for disease recurrence[5].

Where Do We Go From Here?

While effective, the first-generation device to analyze CTCs was difficult to scale up. New devices are continually being developed, including a new herringbone pattern EpCAM-coated chip that has allowed identification of clusters of CTCs in prostate cancer that may provide new and different clues as to how these cells metastasize [6]. Other uses for CTCs have been explored, including methods to define an androgen receptor (AR) signal in these cells that would correlate with AR activity in response to hormonal therapies. Data using this signal in a prostate cancer cell line demonstrated that when AR signaling is "on," the PSA level goes up while the prostate-specific membrane antigen level (PSMA) goes down, and vice versa when AR signaling is "off" after treatment with an antiandrogen agent. If the AR is "mixed," both PSA and PSMA could be up or down. In patients not previously treated with any hormone therapy, the AR signal was largely turned on. This “AR-on” signal in CTCs rapidly switched to “AR-off” with initiation of androgen deprivation therapy (in this study, leuprolide acetate). However in patients with mCRPC, a mixed variety of cell types was seen, with some patients with AR turned on, some off, and a mixture of cells in between. In a study of mCRPC, some patients treated with abiraterone had a decrease in the AR-on fraction, and PSA and CTCs decreased along with the AR-on fraction. Patients who did not respond to therapy had a rise in PSA, CTCs, and AR-off fraction. These studies have demonstrated that AR activity can be measured in prostate CTCs in real time. Measuring AR signaling may be another way to predict and monitor patient response to therapy and guide individualized therapy decisions for patients with PRCA [7].

There is a need for third-generation technology. The ability to analyze single cells is important, including EpCAM-negative CTCs. Future technologies should provide higher sensitivity and specificity and need to be compatible with clinical cytopathology. One new technique under investigation is a CTC-iChip, based on inertial focusing microfluidics. Combining a hydrodynamic cell sorting array, an inertial focusing device, and magnetophoresis, this technology allows cells to emerge in single cell streamlines in a defined location. The magnetically labeled cells can then be deflected and isolated in one output channel, and those not magnetically labeled proceed to another channel. This permits single CTC capture and gene expression profiling. This technique provides high throughput, gentle CTC isolation, and enhanced analyses including high resolution imaging, cytopathology, RNA-ISH, and single cell transcriptional profiling. It may enable clinicians to perform more CTC discovery and individualized cancer care [8]. Looking to the future, continued developments in the analysis of PRCA CTCs may improve development of predictive biomarkers, enhance understanding of the metastatic process, and play potential roles in the early detection of disease and personalization of patient management.

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