EDITORIAL

The Liquid Biopsy for Prostate Cancer 25 Years Later

The concept of the "liquid tumor biopsy" relies on the principle that malignant tumor cells are shed directly into blood, urine and other body fluids. These malignant cells can be captured, counted and examined or their telltale biomarkers can be analyzed using molecular biology techniques leading to more accurate diagnosis, prognosis and treatment.

Relatively crude techniques such as cytology or flow cytometry could detect circulating prostate cancer cells in men with overwhelming metastatic disease as early as the 1970's. This relied on the presence of massive tumor burden and it was impossible to capture and categorize individual cells for further study. Today's technologies allow the capture of relatively small numbers of circulating tumor cells (CTC) that may be present, typically in advanced disease. Whether these CTC are captured using an antibody platform or microfluidic chip these CTC can now undergo further molecular characterization, a critical component in the evolution of the liquid tumor biopsy.

The ability to detect very small numbers of tumors cells and minute circulating biomarkers as hallmarks of cancer can be traced to advances in molecular biology over the last 70 years. The Watson and Crick DNA double helix, the concept of nucleotide base pairs and the discovery of DNA polymerase, an enzyme that replicates DNA, were all critical to the development of what would arguably be the biggest breakthrough in the field of molecular diagnostics and another essential element in the liquid tumor biopsy story. This breakthrough was the technique known as PCR (polymerase chain reaction). PCR allows for the massive expansion of DNA in the laboratory relying upon DNA polymerase. Beyond cancer and basic research investigations, today PCR has become an essential tool in medicine in areas ranging from clinical microbiology to organ transplantation. Most notably the completion of the human genome project with the practice changing discoveries in genomics and gene sequencing relies heavily upon PCR based techniques.

Although a major discovery, DNA polymerase was a very difficult enzyme to work with. That all changed in the 1980's thanks to Mother Nature and the extreme thermophile bacteria Thermus aquaticus. This bacteria lives in hot springs and deep sea hydrothermal vents and has evolved an enzyme that could function optimally around 75°C-80°C, withstanding what would otherwise be protein denaturing temperatures. Nobel Prize laureate Dr. Kary Mullis exploited the fact that this temperature-resistant DNA polymerase, called Taq polymerase in honor of the bacterial species, could withstand extreme heating and cooling cycles to replicate DNA strands. By the late 1980's a commercially produced thermal cycler and other modifications allowed researchers to regulate the PCR reaction to allow rapid and automated amplification of DNA.

An important modification of PCR is RT-PCR (reverse transcriptase PCR) used to detect the expression of a gene. In the area of prostate cancer the major gene of historical interest is PSA. Gene expression in a cell such as prostate cancer is detected by the production of incredibly small amounts of messenger RNA that code for the PSA protein. RT-PCR converts this very small and highly unstable RNA to a larger, more stable and easier to work with complementary DNA. This DNA can be greatly expanded for further study.

My colleagues and I at our new Kimmel Cancer Institute in Philadelphia, the precursor to the Sidney Kimmel Cancer Center at Jefferson, were interested in this relatively new RT-PCR technology and in 1990 began studies using an RT-PCR assay for PSA to detect circulating prostate cancer cells, called micrometastasis at the time. Our initial work, presented at the Mid-Atlantic Section AUA, Williamsburg, VA, in 1992, was published later that year in Cancer Research.[1] Others joined in the investigation of circulating prostate cancer cells in the blood and bone marrow searching for a PSA signal using RT-PCR. There were claims made and subsequently refuted, that a radical prostatectomy should not be done if circulating tumor cells were detected using RT-PCR based on the assumption that metastasis had already occurred. We also determined that prostate cancer cells can shed into the blood and then be rapidly cleared such as immediately after prostate biopsy. Further work investigating the detection of prostate cancer cells using other makers such as PSMA was also begun.

Throughout the 1990's we collaborated with several other US medical centers who shared our interest to determine the assay reproducibility and explore the clinical utility of the RT-PCR PSA test that was assumed to detect circulating prostate cancer cells. While each center could perform the RT-PCR PSA assay, processing and shipping the samples impacted reliability.

Were there other reasons beyond circulating tumor cells that a RT-PCR signal for PSA could be detected in the blood and not represent advanced disease or a tumor cell? With the current interest in the liquid biopsy for prostate cancer, other newly discovered entities such as circulating tumor DNA and nucleic acid containing exosomes might have also been detected by our early RT-PCR blood analysis independent of our concept of a circulating tumor cell.

Thanks to RT-PCR the discovery of shed prostate tumor cells in the urine was also made possible as these cells could be tested for PCA3 and other novel urine based markers. RT-PCR is currently being investigated using whole blood to detect the ARV-7 mutated androgen receptor on CTC. If the abnormal androgen receptor is present, this assay might direct therapy away from androgen biosynthesis pathway inhibitors such as abiraterone and enzalutamide in metastatic castrate resistant prostate cancer (mCRPC).

Beyond prostate cancer it is projected that the liquid tumor biopsy market will grow to nearly 500 million dollars in revenue in 2017 and will continue to expand over the next decade. Since relatively few metastatic prostate cancer lesions are biopsied the liquid biopsy may allow more accurate characterization of metastatic disease when present.

The "liquid tumor biopsy" to identify and further characterize shed tumor cells and unique biomarkers using molecular techniques may provide a basis for personalized treatment strategies in the future and is considered to be a new and novel approach. The liquid biopsy for prostate cancer concept relies heavily on the principles of molecular detection that have been with us for 25 years and the many refinements that have taken place since. This technology will continue to improve and we look forward to the day when these tests become widely available to enhance patient care.

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References

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