AACR Annual Meeting

Finding & Identifying Disseminated Tumor Cells as Biomarkers for Solid Cancers

By Robert H. Carlson

ANAHEIM, CA—The most specific biomarker for solid malignancies might well be disseminated tumor cells found in the serum or elsewhere in the body.

Evidence is mounting that these cells arise very early in disease progression, so their use as biomarkers could point to much greater use of therapies that have lower rates of morbidity and mortality compared with today's standard treatments.

Numerous presentations here at the American Association for Cancer Research's Annual Meeting featured work on finding and identifying disseminated tumor cells, including the following three studies on bone marrow, blood, and feces.

Prostate Cells in Bone Marrow

Researchers from the University of Washington and Fred Hutchinson Cancer Research Center reported that the bone marrow in the majority of men undergoing radical prostatectomy for clinically localized prostate cancer appears to harbor disseminated tumor cells at the time of surgery.

In follow-up biopsies, almost one third of men still had tumor cells in bone marrow after one year.

The data support the hypotheses that tumor-cell dissemination is an early event and that it increases with the aggressiveness of the tumor.

Approximately 500 bone-marrow aspirates from men with various stages of disease were studied, and cells were characterized by immunohistochemistry (IHC), quantitative reverse-transcriptase polymerase chain reaction (RT-PCR), gene expression arrays, and comparative genomic hybridization (CGH) arrays.

Robert L. Vessella, PhD: "Even though the cells are there, that doesn't necessarily mean the patient has a bad prognostic outcome, because the majority of these cells are not able to adapt to the bone marrow or grow in it and cause clinical recurrence of disease. They're just there. Those cells may eventually be eliminated by the immune system, they may fail to adapt, or they may simply remain dormant.

Disseminated epithelial cells were detected in 56% of prostatectomy patients, and prostate-specific antigen (PSA)-positive disseminated tumor cells were detected in 31% of patients.

Detection of disseminated prostate cells and PSA-positive cells was directly associated with increasing pathologic stage, Gleason grade, serum PSA, and tumor volume, and high detection rates correlated with advanced tumors, reported the senior author, Robert L. Vessella, PhD, Professor and Director of the University of Washington's Genitourinary Cancer Research Laboratories.

First author Daniel W. Lin, MD, Assistant Professor of Urology, noted that the cells were found in only 9% of aspirates from men in the study without cancer.

The researchers cannot say with certainty if the disseminated cells are malignant, but the evidence strongly points in that direction, Dr. Lin said. He noted the large number of the cells that stained for PSA, the fact that increasing numbers of cells in marrow correlate with advanced disease, and that the numbers go down after treatment.

He also said patients at high risk of disease progression or those with advanced disease presented with disseminated tumor cells that contained numerous chromosomal alterations.

The data also suggest years of latency, he noted. Among 47 men with no evidence of disease at less than one year after prostatectomy, 25 (53%) still had prostate epithelial cells in the bone marrow.

Among 34 patients tested one to five years after surgery, 10 (29%) had epithelial cells in bone marrow.

And among 31 patients tested more than five years after radical prostatectomy with no indication of disease, the bone marrow in nine (29%) still contained disseminated cells.

"Even though the cells are there, that doesn't necessarily mean the patient has a bad prognostic outcome, because the majority of these cells are not able to adapt to the bone marrow or grow in it and cause clinical recurrence of disease; they're just there," Dr. Vessella said.

"Those cells may eventually be eliminated by the immune system, they may fail to adapt, or they may simply remain dormant.

While it cannot be said that latent cells in bone marrow are responsible for prostate cancer relapse, the numbers are intriguing.

Dr. Lin pointed out that approximately 30% of men in the study had disseminated cells in their bone marrow at five years after surgery for prostate cancer, and that the failure rate after surgery in contemporary series is also about 30%.

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Circulating Tumor-Cell Volume Changes after Breast, Lung Surgery

Researchers in Germany reported quantitative analysis of circulat epithelial cells from lung and br tumors during various steps of ther is feasible using the MAINTR method, which quantitates live epi lial cells in peripheral blood by l scanning cytometry.

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Judah Folkman: Practical Application

The idea of using biomarkers to diagnose cancers years before they can be seen anatomically and treating them before they are symptomatic is very appealing to antiangiogenesis pioneer Judah Folkman, MD, Professor of Pediatric Surgery and Professor of Cell Biology at Harvard Medical School.

"If you have relatively non-toxic drugs, such as the angiogenesis inhibitors, and intersect them with really good biomarkers, you should be able to treat the biomarker and never see the cancer," Dr. Folkman commented, as he viewed these and other poster presentations on novel biomarkers.

"That's what we do with infection now, but before the introduction of antibiotics in the 1930s the physician had to find the abscess to treat it. And in cardiology, it is known that at least one statin drug protects against heart attack years in advance, and cardiologists prescribing those drugs today are basically treating the biomarkers for heart disease.

"Why wait for a heart attack" to treat heart disease?, he asked rhetorically. "But what we [in oncology] are doing is waiting for a cancer to show up, to anatomically locate the cancer [before treating it]."

If reliable biomarkers were available, oncologists might find and treat very early-stage tumors with nontoxic treatments such as the angiogenesis inhibitors, he said, rather than relying on radiation, cytotoxic chemotherapy, or surgery.

Disseminated

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during radiotherapy after surgery.

As with the prostate cancer researchers, the German researchers can only speculate that the circulating epithelial cells are malignant by their response to therapy and their correlation with disease relapse.

"Cancer patients in whom levels of circulating cells rose again after surgery virtually all relapsed," said Katharina Pachmann, MD, Professor of Experimental Hematology and Oncology at Friedrich Schiller-University Jena in Bayreuth, Germany.

Epithelial cells can enter the circulation following punch biopsies in breast cancer but are regularly increased after surgery both in breast and lung cancer, she said. "Without further treatment such cells can remain in the circulation in stable numbers over years."

Katharina Pachmann, MD, reported an unexpected relationship between tumor and blood: While the level of circulating epithelial cells might decrease after surgery, the levels also increased immediately postsurgery in some patients, or during radiotherapy after surgery.

There is speculation that the cells remaining in the circulation for years are constantly renewed from occult metastatic sites, but others think these are resting "stem-type" cells. Chronic inflammation associated with neoplasia might also be a source. Immune deficiency and advancing age could also account for relapse arising from these cells.

Circulating cells are reduced by most chemotherapies, but an increase during therapy is an early predictor of relapse. In breast cancer such increases can be accompanied by an increasing proportion of cells carrying high amplifications of the HER-2/*neu* gene.

High numbers of cells can also be seeded into the circulation upon tumor tissue disintegration during neoadjuvant therapy, Dr. Pachmann said, and these can also remain in the circulation for months after surgery. Normal epithelial cells, which also spike in serum after surgery, are rapidly cleared from the circulation.

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She said future preclinical directions may include pheresis studies. But this analytical method is not suitable for screening, she said, because, for unknown reasons, it is positive for circulating epithelial cells in about 3% of normal, healthy volunteers.

Tumor Cells in Feces

A new method for isolating colonocytes exfoliated into stool and naturally evacuated into feces was also described at the meeting. The isolated cells can be analyzed to detect colorectal cancer arising from any part of the large intestine, including the right side.

The new method has a positive predictive value of almost 90% for colon cancer, versus approximately 10% for the standard fecal occult blood test, said project leader Yasuhiro Matsumura, MD, PhD, Chief of the Investigative Treatment Division of National Cancer Center Research Institute East in Kashiwa City, Japan.

He said colonocytes can be recovered from the central portion of the feces as well as from the surface. In simulation experiments, 35% to 70% of the cells of the colorectal cancer cell line HT-29 employed could be recovered in the feces. He presented data on exfoliated cells in feces collected from 25 patients with colorectal cancer and seven healthy volunteers. Exfoliated colonocytes were examined cytologically and subjected to DNA analysis.

Dr. Matsumura said that prior to this new method, human DNA was difficult to isolate in feces because of the high level of bacterial DNA. For that reason the researchers first searched for colonocytes. From the colonocytes, mutations or abnormalities of the APC, k-ras, p53 genes, and microsatellite instability were identified in 19 of 25 patients with colorectal cancer.

Of these 19, 18 (95%) also showed at least some of the mutations and abnormalities in the colonocytes retrieved from the feces. These mutations were observed in five of six Dukes' Stage A patients and in three of four patients with right-sided colon cancer. None of the genetic abnormalities occurred in the cells retrieved from the fecal specimens of any of the healthy volunteers.

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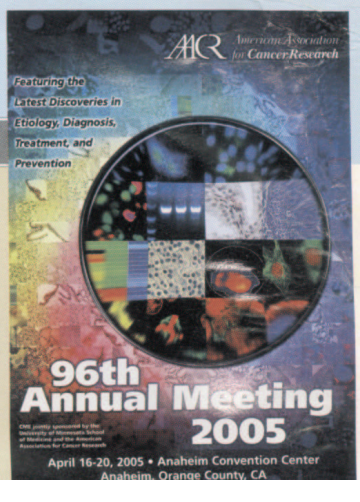

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