Review

Cancer Research

A Need for Basic Research on Fluid-Based Early Detection Biomarkers

Katherine J. Martin[1], Marcia V. Fournier[1], G. Prem Veer Reddy[2], and Arthur B. Pardee[3]

Abstract

Cancer continues to be a major cause of mortality despite decades of effort and expense. The problem reviewed here is that before many cancers are discovered they have already progressed to become drug resistant or metastatic. Biomarkers found in blood or other body fluids could supplement current clinical indicators to permit earlier detection and thereby reduce cancer mortality. *Cancer Res; 70(13); 5203–6. ©2010 AACR.*

The Need for Earlier Detection of Cancer

Progress against cancer has been slow despite decades of effort. Since 1990 the death rate for all cancers combined has decreased by only 1% per year, a decline due to a combination of factors including reductions in tobacco use, improvements in treatment of specific cancers, and successful early detection of a few specific cancers (1). New treatments for leukemia, lymphomas, and testicular cancer have reduced death rates for these cancers, but no magic bullet has emerged. Current anticancer drugs have limited efficacy against advanced cancers. Drugs and surgery can be effective against early tumors, but they usually only briefly extend survival of patients with metastatic cancer. For example, survival for breast cancer patients with early localized disease is 98% as compared with a dismal 27% for those whose cancer has already progressed to distant metastasis at initial diagnosis (2). Although efforts to develop efficacious therapeutics continue, the other side of the coin is the need for improved methods to detect cancers earlier.

Early detection can significantly reduce cancer mortality. Death rates from some major cancers have declined recently because of early detection. In particular, Papanicolau test screening detects and allows removal of presymptomatic cervical cancers and has saved many lives. There have also been partial successes in early detection screening programs for breast, colon, and prostate cancers. Mammography detects 80 to 90% of breast cancers and has reduced risk of death by 15% in all women screened. Unfortunately, mammography also generates a high rate of false positives and detects indolent localized cancers that may not require treatment (3). The effectiveness of screening for colorectal cancer by colonoscopy for people 50 years of age or above reduces incidence as well as mortality, though screening rates remain low (1). Recent declines in prostate cancer deaths were attributed to early detection by prostate-specific antigen (PSA), however, there are still questions about death reduction and overdiagnosis rates (1). Less successful programs do not negate the value of early detection screening. It remains a challenge to researchers to develop screens that overcome current problems and efficiently detect treatable early cancers.

Here we provide an overview of the current state of development of body-fluid biomarkers for cancer detection. Although body-fluid biomarkers have been studied and applied for many years, exciting new discoveries are supported by recent advances in bioinformatics, bioengineering, and high-throughput genomics, transcriptomics, and proteomics technologies.

Body Fluid Biomarkers for Early Detection

Fluid sampling has advantages over imaging as it is widely accepted, readily repeated, convenient, noninvasive, and low cost. Biomarkers in body fluids have the potential to detect a wide variety of primary tumors and metastases located throughout the body. Fluid biomarkers include a variety of components in blood, urine, or other fluids that reflect the presence of a tumor in the body. These include circulating tumor cells (CTC) and macromolecules such as lipids, proteins, RNA, microRNA, and DNA that originate from tumor cells (Fig. 1). In addition to CTCs, other circulating cells being explored include immune, stromal, and endothelial cells. It is particularly exciting that a combination of biomarkers can be more effective than a single biomarker by decreasing false positives and increasing detection of multiple tumor subtypes. Several sources of body fluids can yield biomarkers as described below.

Blood-based mRNA gene signatures

Blood is an excellent fluid in which to find biomarkers. A paradigm for blood biomarkers is the PSA protein test

Authors' Affiliations: [1]Bioarray Therapeutics Inc., Belmont, Massachusetts; [2]Vattikuti Urology Institute, Henry Ford Hospital, Detroit, Michigan; and [3]Dana-Farber Cancer Institute, Boston, Massachusetts

Note: K.J. Martin and M.V. Fournier contributed equally to this work.

Corresponding Authors: Arthur B. Pardee, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115. Phone: 617-547-6786; Email: arthur_pardee@dfci.harvard.edu or Marcia V. Fournier, Bioarray Therapeutics Inc., 61 Carleton Rd., Belmont, MA 02478. Phone: 508-577-0205; E-mail: Marcia.fournier@yahoo.com.

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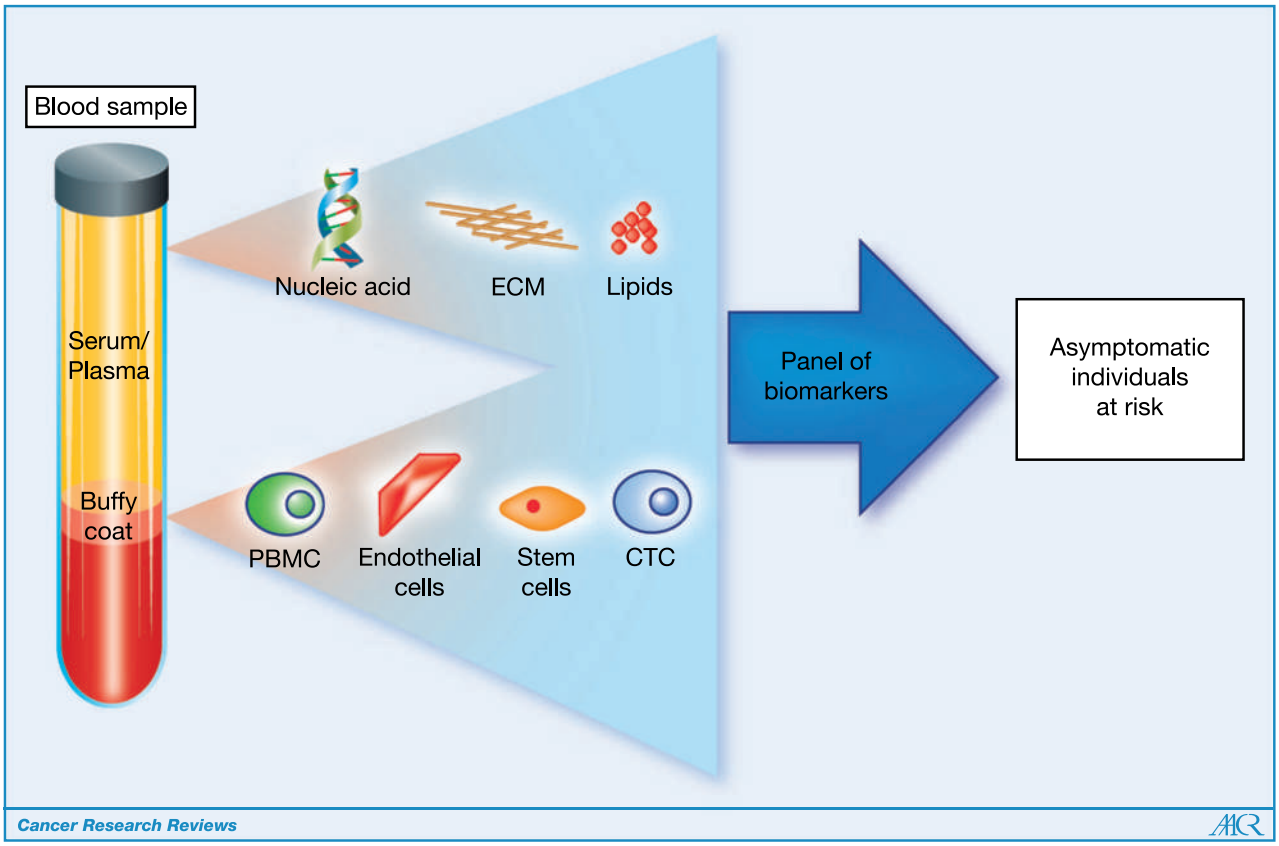

Figure 1. Blood biomarkers for early cancer detection. Biomarker profiles can be obtained from PBMCs, plasma and serum, and selected circulating cells in blood as well as other body fluids including urine, and saliva for identifying asymptomatic individuals at risk. ECM, extracellular matrix fragments.

for prostate cancer. In addition to proteins, mRNAs are promising biomarkers, and microarrays represent a powerful approach for their discovery in blood. A study using custom-spotted arrays published in 2001 identified a signature of 12 genes whose mRNA expression was elevated in peripheral blood mononuclear cells (PBMC) of breast cancer patients (4). Conclusions were limited by low numbers of genes and samples tested. More recent microarray studies have identified additional early detection signatures. A study of PBMCs from lung cancer patients identified a signature of 29 genes that distinguished patients from controls (5). A study of sorted peripheral blood lymphocytes from patients with melanoma and healthy controls identified 17 interferon-stimulated genes that distinguished the groups; findings were confirmed by PCR and functional tests (6). Panels of mRNA biomarkers for early detection have also been identified in bladder, breast, and renal carcinomas (7–9). These studies suggest that blood-based mRNA signatures may be potentially useful for early detection screening of multiple solid tumor types.

Shortcomings of blood mRNA signature studies are notable. It is often not clear if signals originate from tumor cells or PBMCs. Immune cell responses could lead to false positives. Only small scale studies have been done to date and clinical translation will require studies involving larger number of patients.

Circulating tumor cells

CTCs are indicators of cancer and have been extensively reviewed (10, 11). CTC applications are currently limited to following progression and therapy response in patients with metastatic cancer in which five or more CTCs per 7.5 ml blood are associated with disease progression. Several issues must be addressed to allow the application of CTC technology to the early detection of cancer. One challenge is to find highly specific markers able to address a very low signal-to-noise ratio. CTC detection currently relies on a single marker, the epithelial cell surface epitope EpCAM. Although EpCAM is an excellent epithelial cell marker, it is not expressed on all cancer cells (11). Emerging approaches include the use of gene mutations, antibody cocktails, negative selections, and filtrations on the basis of cell size or density (10). Additional studies will determine if CTC counting is applicable to early cancer detection.

Circulating cell-free nucleic acids

The detection of cell-free nucleic acids (RNA and DNA) originating from tumor cells and circulating in serum or

plasma of cancer patients was first reported decades ago (12, 13) and has been verified for a variety of tumor types. The presence of circulating DNAs harboring somatic mutations in growth factor receptors, tumor suppressor genes, and oncogenes has been used as tumor-specific serum biomarkers for cancer cells (13). Recently, Schmidt and colleagues detected mutant DNA in the blood of approximately 50% of patients with early stage colorectal cancer (14).

Preliminary evidence has shown that RNAs are more readily detected in plasma than in the cellular fraction that would contain intact CTCs. Reverse transcriptase (RT)-PCR analysis of the prostate marker KB208E9 mRNA showed a six fold higher level in the plasma than in the buffy coat fraction.[4] Further, serum levels of microRNA-141 distinguished patients with prostate cancer from healthy controls (15). At least four microRNAs (miR-21, miR-210, miR-155, and miR-196a) have been found in plasma and pancreatic cancer tissues but not in healthy controls. Epigenetic modifications including specific DNA methylation changes are increasingly recognized as important in cancer progression and these can also be found in free DNA circulating in cancer patient blood (16).

The clinical value of nucleic acid detection in plasma or serum for early detection has been extensively assessed but results are still inconsistent (17). A limitation is a high rate of false negatives. Although positive results are found in patients with cancer, the absence of circulating nucleic acid does not correlate with the absence of cancer.

Other approaches

Other fluids have been used for early cancer detection. For example, KB208E9 mRNA and other prostate biomarkers are elevated in urine of prostate cancer patients (18) and shown to distinguish metastatic from localized prostate cancer. Saliva has been used to detect oral and breast cancers (19). Hundreds of metabolites have been found in urine, plasma, and tissue specimens of prostate cancer patients. Other promising early detection biomarkers exploit the tumor microenvironment and angiogenic switch by quantifying urinary metalloproteinases and analyzing the platelet angiogenic proteome (20). Circulating endothelial cells and stromal stem cells are also potential future biomarkers for early cancer screening.

Goals of Future Basic Biomarker Research

In this era of highly innovative molecular technologies in the research laboratory, there is a critical need to efficiently translate tests to the clinic. Thousands of articles on biomarkers have been published in the past decade, but few are applied clinically. Recently, a report of a set of promising biomarkers for serum-based detection of ovarian cancer recommended inappropriate clinical applications because of the use of flawed biostatistics (21). Several other promising biomarkers have not been validated in large scale clinical studies. For screening of the general population, the costs of false positives and false negatives are major criteria and minimum acceptable rates can vary depending on the cancer type. For instance, lower false positive rates are acceptable for cancers that require more invasive follow up (e.g., ovarian cancer). There is no formula for selecting successful biomarkers to date, but this should include hypothesis-driven experimental design, robust statistical analysis, representative patient selection, and validation in independent data sets early in biomarker development. Guidelines have been developed for both reporting of results (REMARK) and phases of biomarker development [Early Detection Research Network (EDRN)].

Fluid-based early detection research brings several questions: How early can a cancer be detected by biomarkers? And is the earliest detection of a malignancy always valuable? Some tumors are indolent and never become invasive or life threatening (20), whereas others progress rapidly. Methods to distinguish indolent and aggressive cancers are research topics, but few clinical tests are currently available. Mammaprint and OncoTypeDX gene expression signatures are prototype tests that are applied to breast tumor tissue to identify patients likely to have a good outcome without treatment. However, until there is more experience with and reliance on similar tests, early detection poses the risk of overtreatment. A "watch and wait" approach can be recommended for prostate patients with positive PSA tests and localized cancer, but often patients prefer treatment. The successful implementation of early detection programs will ideally require the codevelopment of tests to distinguish aggressive from indolent tumors.

A further question is posed: What response is appropriate in the situation of a positive body fluid test when the tumor cannot be clinically diagnosed? Experience with the new detection tests will be required to know if systemic treatment is warranted. Small steps will be necessary to validate body fluid biomarkers. For instance, fluid biomarkers could be initially introduced as complimentary tests to confirm positive results from conventional screening methods, and to aid in deciding further treatments.

As with all screening programs, the measure of success will be nothing less than reducing cancer-specific mortality. Determining whether a program is effective could require large (i.e., multithousand patient) clinical trials lasting many years and requiring great expense. Clinical translation is vastly more expensive than basic research. But it is necessary, as is validation of a new drug. Unfortunately, it is much less likely that industry will fund early detection biomarker tests than highly profitable drug development programs.

Given the great expense of clinical trials, it will also be important that the right biomarker sets are taken into clinical trials. Biomarkers are needed for routine early detection screening for all types of cancers. It may be most appropriate if initial trials address frequently lethal cancers for which there are large amounts of data and resources. Archived blood samples with clinical outcome data can be used to test proposed biomarker sets before proceeding to prospective sampling of patients.

[4] V. Bai and P. Reddy, unpublished data.

Methods developed for finding and applying fluid biomarkers for one type of cancer will likely be applicable to other cancer types. In particular, cancers such as pancreatic and ovarian are often discovered too late for effective therapy. It remains to be seen if early detection of these aggressive tumor types will impact mortality rates. In addition to early detection applications, the same fluid biomarker tests would likely also have applications to monitoring therapy response and disease outcome. Improvements in selection of effective therapeutic drugs and surgical procedures could be developed from results.

Conclusions

The application of fluid biomarkers for the early detection of cancers of all types is of significant potential value and deserves a similarly significant share of funding. Roughly 1,000 commercial genetic tests are being developed for medical problems including cancer, but very few biomarker tests are currently applied (22). Although fluid-based biomarkers seem promising, their reliability has not been determined. Assay sensitivity and specificity need to be improved, techniques must be standardized and validated, and biomarkers are not yet closely legally regulated. Pressing financial, political, and cultural issues have the potential to derail anticipated benefits (23).

What directions will cancer research take? We can be confident that general progress and remarkable new discoveries will continue. Techniques for earlier detection are beginning to be developed and could have major impacts. Clinical progress depends on testing novel ideas. Paraphrasing the late Dr. Judah Folkman, "There are only experts on the past, none on the future".

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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