Biomarkers in molecular medicine: cancer detection and diagnosis

Padma Maruvada, Wendy Wang, Paul D. Wagner, and Sudhir Srivastava
Division of Cancer Prevention, National Cancer Institute, Bethesda, MD, USA

BioTechniques 38:S9-S15 (April 2005)

In spite of advances in diagnostics and therapeutics, cancer remains the second leading cause of death in the U.S. Successful cancer treatment depends not only on better therapies but also on improved methods to assess an individual's risk of developing cancer and to detect cancers at early stages when they can be more effectively treated. Current cancer diagnostic imaging methods are labor-intensive and expensive, especially for screening large asymptomatic populations. Effective screening strategies depend on methods that are noninvasive and detect cancers in their early stages of development. There is increasing interest and enthusiasm in molecular markers as tools for cancer detection and prognosis. It is hoped that newly discovered cancer biomarkers and advances in high-throughput technologies would revolutionize cancer therapies by improving cancer risk assessment, early detection, diagnosis, prognosis, and monitoring therapeutic response. These biomarkers will be used either as stand-alone tests or to complement existing imaging methods.

INTRODUCTION

During the past three decades, there has been significant progress in both the understanding and treatment of cancer. However, cancer remains the second leading cause of death in the U.S., and the Director of the National Cancer Institute (NCI) has challenged the cancer community to eliminate suffering and death due to cancer by 2015 (1). Achieving this goal will require not only improved therapies but also improved methods to assess an individual's risk of developing cancer, to detect cancers at early stages when they can be more effectively treated, to distinguish aggressive from nonaggressive cancers, and to monitor recurrence and response to therapy. Improving methods to screen asymptomatic populations for the presence of early stage cancers is a particularly challenging problem. The American Cancer Society has recently recommended various diagnostic tests to screen populations for the early detection of many cancers of high incidence including breast, colon, and prostate (2). However, there are no viable screening methods for other common cancers, such as lung cancer.

While diagnostic, imaging methods can be used to identify individuals with cancer, many are too labor-intensive and expensive for screening large asymptomatic populations. Moreover, some have met with resistance by the general population, as they can be embarrassing or inconvenient, limiting their usefulness for screening this group. Also, diagnostic imaging methods often miss smaller lesions, with the disease not diagnosed until it is in an advanced stage when therapeutic intervention is usually less effective. In the past several years, there has been increasing interest and enthusiasm in molecular markers as tools for cancer detection and prognosis, both as stand-alone diagnostic tools and to complement existing imaging methods and technologies.

In this article, we briefly discuss molecular technologies used for biomarker discovery and analysis and provide examples of how they can potentially be used to identify at-risk individuals and early cancers.

WHY ARE BIOMARKERS USEFUL?

Cancers arise from an accumulation of genetic and/or epigenetic changes that result in alterations of the proteins expressed in the affected cells. The levels of specific proteins can be increased or decreased or their functions and distributions altered by posttranslational modifications. These protein alterations can affect cell metabolism and physiology, cell growth and death, and secretion of molecules that signal other cells and tissues. In cancer research, molecular biomarkers refer to substances that are indicative of the presence of cancer in the body. Biomarkers include genes and genetic variations, differences in messenger RNA (mRNA) and/or protein expression, posttranslational modifications of proteins, and metabolite levels. As the molecular changes that occur during tumor progression can take place over a number of years, genomic, proteomic, and metabolomic biomarkers can all be potentially used to detect cancer, determine prognosis, and monitor disease progression and therapeutic response (Figure 1).

Traditionally, biomedical research has been hypothesis-driven; investigators put forth hypotheses and design experiments to test them. Recent advances in high-throughput technologies have given rise to more technology-driven research. Rather than putting forth a hypothesis, investigators apply high-throughput methods to biological systems and look for interesting results that could lead to hypothesis generation for further testing. For example, using microarrays containing thousands of different cDNAs, it is possible to look for differences in gene expression in cancerous versus normal tissue. Both hypothesis-driven and technology-driven approaches are applicable to biomarker discovery.

PLATFORMS FOR BIOMARKERS ANALYSIS

Genomic Technologies

Genomic technologies allow the determination and monitoring of genetic factors that underlie carcinogenic transformation and genetic alterations caused by environmental agents. Commonly used genomic technologies include DNA microarrays, PCR-based assays, and fluorescence in situ hybridization (FISH). Advantages of these genomic approaches include the existence of a number of high-throughput robust assay methods and the ability to amplify specific DNAs and RNAs that may exist in very low concentrations in the specimens. DNA-based biomarkers include genetic mutations, loss of heterozygosity (LOH), microsatellite instability (MSA), and DNA methylation. RNA-based biomarkers are mostly mRNAs found in tissues and bodily fluids.

DNA microarrays have revolutionized the field of molecular biology by allowing the simultaneous determination of the expression of thousands of genes, making it possible to determine gene expression profiles under a variety of biological conditions. By comparing the relative levels of mRNA for thousands of genes in normal and cancerous tissues, it is possible to identify biomarkers that are differentially expressed in malignant tissues and to classify them, including lymphoma, leukemia, lung, and breast tumors (3). Once a panel of genes has been identified for a particular cancer, the challenge is to translate this information into a robust assay that can be efficiently applied in the clinic. Many companies are

beginning to commercialize these technologies into meaningful clinical tests (4).

Oligonucleotide-based microarrays are used to study genetic mutations and polymorphisms and are used for genome-wide mutation searches. One advantage of oligonucleotide-based microarrays is the ability to amplify the DNA signal from even suboptimal samples.

A disadvantage of DNA microarrays is that they provide information only on relative gene expression levels and not quantitative measurements of mRNA. Real-time PCR is an alternate technology that provides quantitative measurements of gene expression and allows for amplification and analysis with little sample processing and no radioactivity (5). This technique can analyze multiple genes simultaneously and is a powerful multiplexed tool for analyzing target genes from microdissected tissue. Once the target genes or mRNAs are identified and characterized, real-time PCR may emerge as the method of choice for many genomic analyses. Many PCR-based technologies are being developed for genetic screening (6).

Proteomic Technologies

The term proteomics was originally coined to describe large-scale, high-throughput separations and identifications of proteins, but has been expanded to include functional and structural analyses of proteins. Proteomics provides information that is both complementary and distinct from that provided by genomics. Protein-based biomarkers include alterations in the levels and posttranslational modifications of proteins found in tissues and bodily fluids. Advantages of proteomic approaches include the existence of established and quantifiable assay methods. Indeed, most cancer biomarkers used today in the clinic are antibody-based tests for proteins in sera [e.g., prostate-specific antigen (PSA) for prostate cancer and cancer antigen-125 (CA-125) for ovarian cancer].

Hypothesis-driven research has provided a wealth of information on proteins that play significant roles in carcinogenesis and whose abnormal expressions may be useful as biomarkers for cancer. However, the number of clinically useful biomarkers that have resulted from this approach is quite low, and many investigators are exploring high-throughput technologies to expedite the discovery process. Technology-driven high-throughput proteomic approaches include 2-dimensional gel electrophoresis (2-DE) and various forms of mass spectrometry (MS). Extracts from control and cancerous tissues or cells are fractionated by 2-DE, and proteins, whose intensities are significantly increased or decreased in disease tissues, are identified using MS. For example, 2-DE was recently used in combination with MS to identify proteins that are specifically overexpressed in a variety of cancers, including ovarian and gastric cancers (7,8). The limitations of the 2-DE approach are well known and include lack of reproducibility, low resolution, and the need for relatively large amounts of sample. Even with recent modifications, 2-DE is a relatively low-throughput methodology with limited clinical applicability.

A number of newer methods for large-scale protein analysis are being used or are under development. Several of these rely on MS and database interrogation, and include fractionation of protein extracts by liquid chromatography (LC) followed by identification of proteins or peptides by MS. Comparative MS methods, which rely on labeling with different isotopes, have been developed to determine the relative amounts of peptides or proteins in two different samples. Although these comparative MS techniques are useful for determining proteins that are differently expressed in control versus disease, they are expensive, low-throughput, and not suitable for routine clinical use.

One of the more widely used proteomic technologies is surface-enhanced laser desorption/ionization time-of-flight MS (SELDI-TOF MS). SELDI-TOF MS has the potential to be high-throughput and adaptable to clinical use. Protein fractions or bodily fluids are spotted on chromatographic surfaces that selectively bind a subset of proteins (ProteinChip® Arrays; Ciphergen Biosystems, Fremont, CA, USA), and the bound proteins are ionized and analyzed by TOF MS. This method has been used to identify cancer biomarkers, including the α-chain of haptoglobin (Hp-α) for ovarian cancer and α-defensin for bladder cancer (9,10). Investigators are also using SELDI-TOF MS to acquire proteomic patterns from bodily fluids. These complex patterns are analyzed using pattern recognition algorithms to identify a set of peaks that can be used to distinguish cancer from control. An interesting aspect of this approach is that the identification of these proteins or peptides may not be necessary for the development of a clinical assay; a SELDI-TOF MS protein profile by itself may be sufficient for screening. For example, SELDI-quadruple-TOF MS high-resolution profiles have been reported to identify patients with stage I ovarian cancer with 100% sensitivity and specificity (11). Similar but less dramatic results have been reported for other types of cancers (12,13). Recently, other proteomic approaches, such as matrix-associated laser desorption/ionization time-of-flight MS (MALDI-TOF MS), have been applied to clinical practice for cancer detection (14,15).

It is uncertain whether protein profiling will prove to be as valuable a diagnostic tool as the initial papers suggest. Factors, such as interfering or high-abundant proteins in the sample, variations in diet, or inflammation, which can influence the protein patterns and peak intensity, need to be taken into consideration. Other factors, such as sample quality, variability between instruments, inter-lab variations, and differences in chip composition from separate batches and companies, can all influence the reproducibility and resolution of the patterns. There is also need for robust algorithms that can effectively detect molecular patterns reproducibly and consistently. Currently many labs, including NCI's Early Detection Research Network (EDRN)-sponsored labs, are conducting validation studies to address these issues.

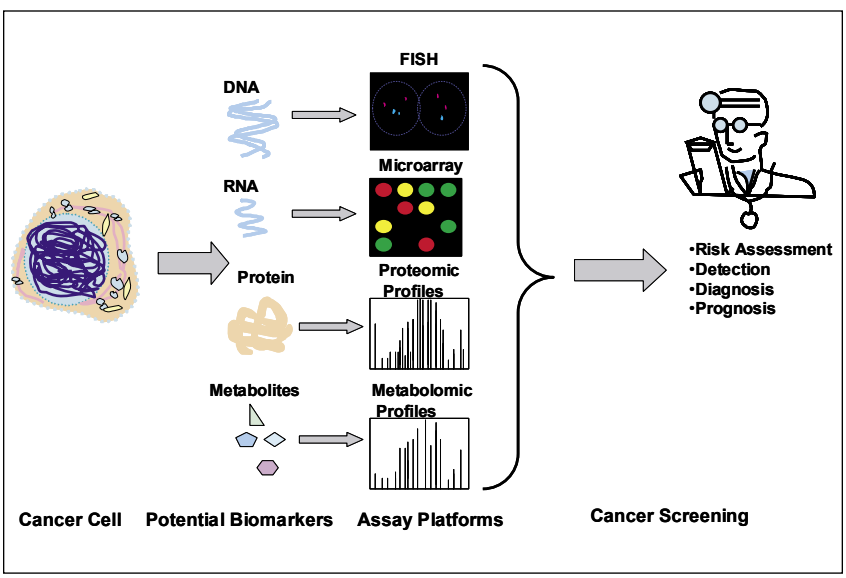

Figure 1. Approaches for biomarker discovery for clinical application. Cancer cell signatures can serve as biomarkers in clinical cancer research for risk assessment, detection, diagnosis, and prognosis, and for determining response to treatment. These biomarkers can be analyzed by high-throughput genomic, proteomic, and metabolomic technologies.

Another proteomic approach gaining attention for molecular screening is protein arrays composed of either recombinant proteins or antibodies. These protein arrays are similar to DNA microarrays in their layout and utilize the principles of immunoassays for detection of tumor antigens. Robust platforms with high analytical sensitivity enable simultaneous detection of several tumor-specific antigens. Specific antigen-antibody interactions make this approach sufficiently precise to identify cancer-specific antigens and enable accurate diagnosis of cancer. Its portability and throughput make it attractive as a screening tool.

Metabolomic Technologies

Metabolomics refers to the study of metabolites present in cells, tissues, and bodily fluids. The potential usefulness of metabolomics for detecting and monitoring cancer is that the identities, concentrations, and fluxes of these molecules are the final products of interactions between gene expression, protein expression, and the cellular environment. The limited number of metabolites and metabolic products makes them suitable for analysis by high-throughput methods. Carcinogenic transformation often involves changes in cellular metabolites, and metabolites of environmental toxins that play an important role in carcinogenic transformation are detectable in bodily fluids. Metabolomic approaches use analytical techniques such as nuclear magnetic resonance spectroscopy (NMR), high-performance liquid chromatography (HPLC), gas-liquid chromatography (GLC), and MS to measure populations of low-molecular-weight metabolites. Advanced statistical and bioinformatic tools are then employed to maximize the recovery of information and to interpret the large data sets. An advantage of the metabolomic approach is that it is possible to interrogate more than one type of molecule (lipids, carbohydrates, nucleotides) at a time, giving a better description of cellular events. For example, oxidative damage may change cellular antioxidant status, resulting in differences in a variety of cellular pro-oxidant and antioxidant molecules. It is, therefore, always advantageous to analyze all the molecules and pathways. Metabolomic patterns may be influenced by the same factors as proteomic analyses. Recently, metabolomic approaches have been utilized to study the metabolic changes of hypoxia inducible factor-1 deficient tumors (16). The potential of metabolomics for cancer detection is just beginning to be explored.

Multiplex Approaches

A limitation of many, if not all, currently used cancer biomarkers, is that they do not detect all individuals with cancer (false negatives). This results from both the progressive nature of cancer and its heterogeneity. Cancer is not a single disease but rather an accumulation of several events, genetic and epigenetic, arising in a single cell over a period of time. The problem of biomarkers indicating the presence of cancer when none is present (false positives) results because these biomarkers are not uniquely present in tumors. With no single biomarker providing 100% sensitivity and specificity, there is a need for an ensemble of biomarkers to enable molecular screening to reduce false positive and false negative cases. Consequently, methods that allow for the simultaneous measurement of several biomarkers are needed.

Sophisticated molecular technologies that can simultaneously identify a variety of promising markers are employed in the discovery phase. The same technologies may be used for clinical application. However, often as a biomarker progresses from discovery to validation and to subsequent clinical application, its measurement needs to be adapted to various platforms. Not all technologies are robust enough to pass through all the phases of biomarker development. For large-scale screenings, technologies need to be robust, flexible, and cost-efficient. Multiplex platforms allow for simultaneous analysis of several different biomarkers and are, therefore, attractive platforms for screening. For example, quantitative multiplex-methylation-specific PCR (QM-MSP) is a highly sensitive method that can determine the hypermethylation status of multiple genes, such as RASSF1A, TWIST, Cyclin D2, and HIN1, in a single tube (17). Using Invader® assays (Third Wave Technologies, Madison, WI, USA), it is possible to simultaneously quantitate both RNA and DNA (6). LabChip® technology in combination with the 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) enables the analysis of DNA, RNA, protein, and cellular substances from a single sample (18). These approaches decrease the laborious sample preparation times. Another revolution in molecular detection is microfluidics technology that allows detection of molecules in suspension using lasers and fluorescent dyes (18). It is now possible to simultaneously perform 100 bioassays in a single reaction with high sensitivity. Such approaches also require analytical tools to analyze the multidimensional, high-throughput data (19–21). While the optimization of the technologies can be very complex, these multiplex technologies offer great promise for molecular screening.

PERFORMANCE CHARACTERISTICS OF BIOMARKERS IN SCREENING

Imaging is the most frequently used method for cancer screening. Currently, mammography for breast cancer, colonoscopy for colon cancer, and X-ray for lung cancer are the preferred screening methods. While mammography is effective as a frontline screening tool, its sensitivity significantly decreases in dense and heterogeneously dense breasts. It misses almost half of palpable tumors in extremely dense breasts (22). Such uncertainties could result in unnecessary repeated exposure to radiation, surgical interventions, and psychological and emotional stress due to prophylactic mastectomies. Magnetic resonance and ultrasound imaging are better able to detect malignant and invasive cancers compared to mammography. These imaging strategies have been thought to improve the shortcomings of mammography screening. However, several research groups have shown that these sensitive screening methods result in overdiagnoses, leading to increased recall and negative open surgical biopsy rates in the U.S. (22,23). Molecular screening methods in combination with these imaging techniques may help alleviate this situation.

Biomarkers used for screening need to be able to detect early stage disease with high precision and sensitivity. Ideally, these biomarkers should be detected in specimens that can be collected by noninvasive means. Before any biomarker can be successfully employed in the clinic, its statistical and analytical performance characteristics must be validated. Various factors that influence the utility of the biomarker, such as age, diet, sex, and other associative factors need to be carefully considered in the initial stages of screening study designs and methods. Recently, various aspects of biomarker development have been discussed to guide molecular screening (24).

Statistical characteristics of cancer screening tests include sensitivity and specificity. In the context of cancer screening, sensitivity refers to the proportion of individuals with cancer who test positive, and specificity refers to the proportion of individuals without cancer who test negative. An ideal screening test would have 100% sensitivity and specificity; everyone with cancer would have a positive test, and everyone without cancer would have a negative test. Unfortunately, most of the recommended screening tests have less than ideal performance characteristics, leading to a number of false positives and false negatives. Digital rectal examination (DRE) is the most common screening test for prostate cancer. However, its sensitivity and specificity vary widely and often leads to unreliable conclusions. The PSA test attempts to address these inadequacies. The normal range of PSA in serum is between 0–4 ng/mL. A serum PSA test value above this range would indicate

the possible presence of prostate cancer. However, PSA is not specific to prostate cancer. It has a sensitivity of 70% and a specificity of 59%–97%, varying widely among different studies (25–28). Decreasing the PSA cutoff value to below 4 ng/mL would result in improved sensitivity, but specificity would decease, resulting in an increase in false positives. Even when PSA is used in combination with DRE, false positives are not eliminated. In a study conducted in the UK, PSA and DRE were used as frontline screening tools, and men with an abnormal result were examined using transrectal ultrasound (TRUS) imaging. Even with this effective screening design, 6% of the men had biopsies, but only 1.7% had prostate cancer (29). Similar results have been reported in other screening trials of asymptomatic men (30).

Even when a screening method provides high specificity and sensitivity, it may not be useful for screening the general population if the cancer has low prevalence. For example, if the cancer's prevalence is 1% and the test has 100% sensitivity and 95% specificity, only 17 out of 100 people with a positive test actually have cancer. The significance of this problem is obvious when one considers that the prevalence of ovarian cancer in the general population is about 0.04%.

The final step in evaluating a biomarker for screening is a large-scale population study that evaluates not only the utility of the biomarker for cancer detection but also the overall impact of screening on the population. These trials are time-consuming and expensive, and to date, few have been undertaken. For example, a 12-year screening trial for prostate, lung, colon, and ovarian cancer (PLCO) is still ongoing. Effective molecular tools that enable more accurate screening and flexible study designs may accelerate this process.

BIOMARKERS IN CANCER SCREENING

The type of biomarker used depends both on the application (exposure, risk assessment, early detection, prognosis, or response to therapy) and the availability of appropriate assay methods. While some markers give information on the presence of cancer, others provide information on the risk of developing cancer or the likelihood of an individual responding to a particular therapy. Some examples of biomarkers used in cancer research are listed in Table 1.

Biomarkers of Exposure

Various environmental agents, such as smoking, asbestos, radiation, and other toxic agents, play important roles in carcinogenic transformation. Analysis of these compounds or their metabolites in biological fluids can provide a measure of the extent of exposure and, thereby, the risk of developing disease. Cotinine, which results from cigarette smoke, can be detected in urine and serum, and 1-hydroxypyrene, resulting from exposure to polycyclic aromatic compounds, and aflatoxin M_1, resulting from dietary exposure, can be detected in urine (31–33). Environmental agents can cause oxidative DNA damage and alterations in the DNA repair enzymes. Monitoring DNA adducts, single strand DNA breaks, chromosomal aberrations, sister chromatid exchanges and micronuclei, and other cytogenetic effects can provide useful information on carcinogen-induced damage. For example, the measurement of chromosomal aberrations in peripheral lymphocytes is a sensitive monitor of exposure to ionizing radiation (34). Although biomarkers of exposure may not provide direct information for the presence of disease, they do provide information on the extent of increased risk of developing cancer.

Biomarkers of Risk

The majority of hereditary cancers, such as hereditary breast and ovarian cancer (HBOC), hereditary non-polyposis colon cancer (HNPCC), and familial adenomatous polyposis (FAP), involve germline mutations that put individuals at increased risk of developing these cancers (35,36). For example, the breast cancer-associated *BRCA-1* gene encodes a 220-kDa nuclear protein that responds to DNA damage by participating in cellular pathways responsible for DNA repair, mRNA transcription, cell cycle regulation, and protein ubiquitination (37). *BRCA-1/2* mutations are used to determine the risk of women with a family history of breast cancer of developing breast cancer (38). At least 10% of breast tumor patients carry *BRCA* mutations and are at increased risk for developing cancer early in their life. *BRCA* carriers diagnosed with cancer in one breast are at high risk of developing high-grade tumors in the contralateral breast compared to the general population (39).

Many genetic tests are currently used to detect mutations in DNA for cancer risk assessment and for genes that predispose an individual to inherited cancer syndromes (40–43). The study of chromosomal aberrations and translocations frequently rely on molecular imaging techniques that are often time-consuming and lack portability. Recently it has been possible to identify the subgroups of patients at risk for developing leukemia by investigating myelodysplastic syndromes (MDS) karyotypic aberrations using FISH (44).

Markers of susceptibility provide information to identify high-risk individuals and stratify populations. Once a high-risk cohort has been identified, it will be possible to monitor these individuals for the development of cancer. A problem associated with biomarkers of risk and exposure is that mere presence of such a biomarker does not mean that an individual will subsequently develop cancer. For example, although infection with *Helicobacter pylori* increases the risk of developing gastric cancer, not all the individuals that are positive for an *H. pylori* infection develop gastric cancer (45). Similarly, detection of *APC* mutations and polyps in FAP indicates that an individual is at risk of developing colon cancer. It does not, however, determine if that individual is in fact going to develop the disease (46). Such screening test results can cause anxiety and undesirable sociopsychological outcomes. Other concerns with screening for risk are unethical genetic and racial discrimination and breaches of privacy (35).

Biomarkers of Early Detection

Carcinogenic transformation often results in secretion of elevated levels of biomarkers or abnormal molecules. Presence of such markers in bodily fluids can give information on the course of the disease. Studies have shown that specific proteins are overexpressed in a variety of cancers (47–51). For example, PSA is secreted by prostate tissue and has been approved for the clinical management of prostate cancer (52). CA-125 is a protein that is secreted by ovarian tissue and is recognized as an ovarian cancer-specific protein (53). Identification of such biomarkers is of great interest as early detection of cancers may enhance treatment options, thereby increasing survival rates and providing for better disease management. Although biomarkers have great potential as effective screening tools, most of those currently available are far from ideal, and more research is needed in this area to develop and validate biomarkers for early detection or to develop panels of similar biomarkers.

Biomarkers in Prognosis and Treatment

Biomarkers are also used for cancer prognosis, prediction of recurrence, and monitoring drug response. Prognostic markers include genetic polymorphisms, mutations, and alterations in the protein expression. Breast tumors in carriers of *BRCA-1* mutations are characterized by poor differentiation, high proliferation index, aneuploidy, and negative estrogen receptors, whereas tumors in carriers of *BRCA-2* mutations are similar to sporadic breast tumors (39). Despite the adverse phenotype and negative estrogen

Table 1. Examples of Biomarkers in Cancer Research

Biomarker	Technology	Biomarker Application and Cancer Affected Organ	References
Genomic			
BRCA-1 and *BRCA-2* mutations (blood)	DNA sequencing	Risk assessment and prognosis (breast)	38
Chromosomal damage (blood, white cells)	Comet assay, micronucleus assay	Diagnosis and risk assessment (acute myeloid leukemia, acute lymphoma)	59
Osteopontin (blood)	Microarray	Detection, diagnosis, and prognosis (ovaries)	60
Ras mutations (blood)	Short oligonucleotide mass analysis (SOMA), PCR primer-introduced restriction with enrichment for mutant alleles (PCR-PIREMA)	Risk assessment (colon and lung)	61,62
Her-2/neu (tissue, serum)	FISH, PCR	Prognosis and treatment (breast)	63
Glutathione S-transferase (GSTP1) polymorphisms (blood)	PCR restriction fragment-length polymorphism assay (PCR-RFLP assay)	Risk assessment, prognosis, and treatment (breast and prostate)	64,65
Cytochrome P450 mutations *CYP3A4* polymorphisms (blood)	DNA sequencing	Risk and assessment and prognosis (prostate and breast)	66
Epigenomic			
Methylation markers RASSF1A, TWIST, cyclin D2, and HIN1 (blood)	Methyl-specific PCR	Diagnosis (breast)	17
Proteomic			
PSA (serum)	Immunoassay	Detection, diagnosis, and prognosis (prostate)	52
CA-125 (serum)	Immunoassay	Detection, diagnosis, and prognosis (ovary)	53
Cancer antigen-19 (CA-19) (serum)	Immunoassay	Diagnosis and prognosis (pancreas and colon)	67,68
Carcinoembryonic antigen (CEA) (serum)	Immunoassay	Detection, diagnosis, and prognosis (colon, lung, and breast)	68–70
Protein profiling (serum)	SELDI, MALDI	Detection and diagnosis (multiple organs)	11–13
Her-2/neu (tissue and serum)	Immunohistochemistry (IHC) and immunoassay	Prognosis and treatment (breast)	58
EGFR (serum)	Immunoassay	Prognosis and treatment (lung)	57
Haptoglobin (serum)	IHC, immunoassay	Diagnosis, treatment response (lung, colon, and breast)	7,15,71,72
Metabolic			
Cotinine (serum, urine)	High-performance liquid chromatography (HPLC)	Exposure (lung)	32
1-Hydroxypyrene (urine)	HPLC	Exposure (lung)	31
Aflatoxin M1 (blood)	HPLC	Exposure (liver)	33
Deoxynivalenol (DON) (serum)	Mass spectrometry	Exposure and risk assessment (esophagus)	73
Lysophosphatidic acid (LPA) (serum)	Mass spectrometry	Detection, diagnosis, and prognosis (ovary)	74
Metabolomic			
Metabolomic profiling (serum)	1H NMR spectroscopy	Early detection and diagnosis (ovary)	75
Stable isotope-based dynamic metabolic profiling (SIDMAP) of glucose (tumor cells)	Mass spectrometry	Drug discovery and treatment (pancreas)	76
Metabolic profiling (tumor)	1H NMR spectroscopy	Diagnosis (brain)	77

Genomic, epigenomic, proteomic, and metabolic biomarkers can be used in cancer research. The approaches for biomarker analysis and their application to cancer research are given. The relevance of the biomarker to a cancer type and the nature of the specimen in which the biomarker can be analyzed are given in parentheses.

receptor status, *BRCA-1* carriers have good prognoses compared to *BRCA-2* carriers and sporadic breast cancers and have better overall survival (54–56). Genetic polymorphisms and mutations may predict an individual's response to a drug and help the physician determine a suitable treatment regimen. For example, epidermal growth factor (EGF) receptor mutations have recently been shown to correlate with clinical response to gefitinib (Iressa®) (57). FISH-based clinical assays are used to predict the sensitivity to Herceptin® (trastuzumab) treatment for breast cancer (58). In some instances, a single biomarker can be used in more than one clinical application. For example, *BRCA-1/2* mutations can be used both in risk assessment and as prognostic markers. PSA is used both to screen for prostate cancer and to monitor for recurrence of prostate cancer. CA-125 has been effectively used for monitoring ovarian cancer treatment and as a diagnostic marker.

COLLABORATION IN BIOMARKER RESEARCH

A large and significant challenge facing the further development of molecular screening tests is to move biomarkers from the laboratory to the clinic. The complexity of taking a biomarker from its initial discovery through to an accepted clinical tool is beyond the scope and expertise of any individual laboratory and will require the commitment and collective efforts of various organizations and individuals.

Investigators from academia, government, and industry need to be committed to developing novel biomarkers with improved performance characteristics. Promising biomarkers need optimization for their use in clinical screening, and novel technologies need to be carefully validated. This will require coordination among biologists, clinicians, statisticians, and bioinformatic professionals. Industrial involvement from the initial stages should improve the likelihood of commercialization. NCI's Early Detection Research Network, a consortium of basic and clinical laboratories, is committed to bringing novel biomarkers from their initial discovery to clinical practice by creating the necessary infrastructure for the validation of these markers and developing standards and reference materials.

The American Cancer Society publishes annual guidelines for cancer screening. These guidelines are intended to enhance knowledge of early detection modalities, to improve decision-making with patients, and to draw attention to the unmet potential for early detection programs to reduce cancer mortality (2). Interaction between investigator-networks, cancer societies, and patient advocacy groups will help establish realistic goals for molecular screening. Another important aspect of public forums is to bring awareness to health care providers and other professionals involved in screening. Cancer societies and patient advocacy groups have the responsibility to educate healthcare professionals about the recent advances in screening.

CONCLUSIONS

It seems unlikely that in the near future molecular markers will replace diagnostic imaging, but rather they will complement imaging in the screening process. Noninvasive molecular markers combined with diagnostic imaging can provide better ammunition to fight cancer by facilitating the screening process and enabling earlier detection. For example, one can envision that a relatively inexpensive biomarker test could be used to screen large populations and that positive tests would be confirmed by more expensive and accurate imaging tests. Such a scenario would require careful patient counseling so as not to cause undue anxiety if the biomarker test is positive. It is hoped that panels of biomarkers will improve overall test sensitivity and specificity, thereby, reducing the numbers of false positive and false negative results.

A common and valid criticism of biomarkers for early detection is that early detection may not necessarily result in reduced morbidity or mortality. Even for those accurately diagnosed as having cancer, the incremental benefit of early detection may be outweighed by adverse side effects of the treatment. While these are valid concerns, development of methods to more accurately detect cancers early will reduce the number of incorrect results and will stimulate the search for improvements in therapeutic strategies, leading to better management of the disease and, thereby, reduce suffering and death due to cancer.

COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

REFERENCES

1. **Miller, M.** 2003. 2015: A Target Date for Eliminating Suffering and Death Due to Cancer. NCI Bench Marks vol. 3.
2. **Smith, R.A., V. Cokkinides, and H.J. Eyre.** 2004. American Cancer Society guidelines for the early detection of cancer, 2004. CA Cancer J. Clin. *54*:41-52.
3. **Zakroff, J.** 2003. Microarrays prove to be effective clinical diagnostic tools. IVD Technology:11-12.
4. **Zakroff, J.** 2003. DNA chips enter the clinical market. IVD Technology:10-11.
5. **Bernard, P.S. and C.T. Wittwer.** 2002. Real-time PCR technology for cancer diagnostics. Clin. Chem. *48*:1178-1185.
6. **Jain, K.** 2002. From molecular diagnostics to personalized medicine. The IBC Workshop. Expert Rev. Mol. Diagn. *2*:299-301.
7. **Ahmed, N., G. Barker, K.T. Oliva, P. Hoffmann, C. Riley, S. Reeve, A.I. Smith, B.E. Kemp, et al.** 2004. Proteomic-based identification of haptoglobin-1 precursor as a novel circulating biomarker of ovarian cancer. Br. J. Cancer *91*:129-140.
8. **Wang, K.J., R.T. Wang, and J.Z. Zhang.** 2004. Identification of tumor markers using two-dimensional electrophoresis in gastric carcinoma. World J. Gastroenterol. *10*:2179-2183.
9. **Vlahou, A., P.F. Schellhammer, S. Mendrinos, K. Patel, F.I. Kondylis, L. Gong, S. Nasim, and G.L. Wright, Jr.** 2001. Development of a novel proteomic approach for the detection of transitional cell carcinoma of the bladder in urine. Am. J. Pathol. *158*:1491-1502.
10. **Ye, B., D.W. Cramer, S.J. Skates, S.P. Gygi, V. Pratomo, L. Fu, N.K. Horick, L.J. Licklider, et al.** 2003. Haptoglobin-alpha subunit as potential serum biomarker in ovarian cancer: identification and characterization using proteomic profiling and mass spectrometry. Clin. Cancer Res. *9*:2904-2911.
11. **Conrads, T.P., V.A. Fusaro, S. Ross, D. Johann, V. Rajapakse, B.A. Hitt, S.M. Steinberg, E.C. Kohn, et al.** 2004. High-resolution serum proteomic features for ovarian cancer detection. Endocr. Relat. Cancer *11*:163-178.
12. **Zhu, X.D., W.H. Zhang, C.L. Li, Y. Xu, W.J. Liang, and P. Tien.** 2004. New serum biomarkers for detection of HBV-induced liver cirrhosis using SELDI protein chip technology. World J. Gastroenterol. *10*:2327-2329.
13. **Wilson, L.L., L. Tran, D.L. Morton, and D.S. Hoon.** 2004. Detection of differentially expressed proteins in early-stage melanoma patients using SELDI-TOF mass spectrometry. Ann. NY Acad. Sci. *1022*:317-322.
14. **Howard, B.A., Z. Zheng, M.J. Campa, M.Z. Wang, A. Sharma, E. Haura, J.E. Herndon II, M.C. Fitzgerald, et al.** 2004. Translating biomarkers into clinical practice: prognostic implications of cyclophilin A and macrophage migratory inhibitory factor identified from protein expression profiles in non-small cell lung cancer. Lung Cancer *46*:313-323.
15. **Bharti, A., P.C. Ma, G. Maulik, R. Singh, E. Khan, A.T. Skarin, and R. Salgia.** 2004. Haptoglobin alpha-subunit and hepatocyte growth factor can potentially serve as serum tumor biomarkers in small cell lung cancer. Anticancer Res. *24*:1031-1038.
16. **Griffiths, J.R. and M. Stubbs.** 2003. Opportunities for studying cancer by metabolomics: preliminary observations on tumors deficient in hypoxia-inducible factor 1. Adv. Enzyme Regul. *43*:67-76.
17. **Fackler, M.J., M. McVeigh, J. Mehrotra, M.A. Blum, J. Lange, A. Lapides, E. Garrett, P. Argani, et al.** 2004. Quantitative multiplex methylation-specific PCR assay for the detection of promoter hypermethylation in multiple genes in breast cancer. Cancer Res. *64*:4442-4452.
18. **Jain, K.** 2002. The role of protein-chip technology in molecular diagnostics. IVD Technology:15-30.
19. **Pittman, J., E. Huang, H. Dressman, C.F. Horng, S.H. Cheng, M.H. Tsou, C.M. Chen, A. Bild, et al.** 2004. Integrated modeling of clinical and gene expression information for personalized prediction of disease outcomes. Proc. Natl. Acad. Sci. USA *101*:8431-8436.
20. **Pittman, J., E. Huang, J. Nevins, Q. Wang, and M. West.** 2004. Bayesian analysis of binary prediction tree models for retrospectively sampled outcomes. Biostatistics *5*:587-601.
21. **Liang, M., A.W. Cowley, Jr., and A.S. Greene.** 2003. High throughput gene expression profiling: A molecular approach to integrative physiology. J. Physiol. *554*:22-30.
22. **Berg, W.A., L. Gutierrez, M.S. Nessaiver, W.B. Carter, M. Bhargavan, R.S. Lewis, and O.B. Ioffe.** 2004. Diagnostic accuracy of mammography, clinical examination, US, and MR imaging in preoperative assessment of breast cancer. Radiology *233*:830-849.
23. **Smith-Bindman, R., P.W. Chu, D.L. Miglioretti, E.A. Sickles, R. Blanks, R. Ballard-Barbash, J.K. Bobo, N.C. Lee, et al.** 2003. Comparison of screening mammography in the United States and the United Kingdom. JAMA *290*:2129-2137.
24. **Sullivan Pepe, M., R. Etzioni, Z. Feng, J.D. Potter, M.L. Thompson, M. Thornquist, M. Winget, and Y. Yasui.** 2001. Phases of biomarker development for early detection of cancer. J. Natl. Cancer Inst. *93*:1054-1061.
25. **Bangma, C.H., D.E. Grobbee, and F.H. Schroder.** 1995. Volume adjustment for intermediate prostate-specific antigen values in a screening population. Eur. J. Cancer *31A*:12-14.
26. **Gann, P.H., C.H. Hennekens, and M.J. Stampfer.** 1995. A prospective evaluation of plasma prostate-specific antigen for detection of prostatic cancer. JAMA *273*:289-294.
27. **Gillatt, D. and J.M. Reynard.** 1995. What is the 'normal range' for prostate-specific antigen? Use of a receiver operating characteristic curve to evaluate a serum marker. Br. J. Urol. *75*:341-346.
28. **Lepor, H., R.S. Owens, V. Rogenes, and E. Kuhn.** 1994. Detection of prostate cancer in males with prostatism. Prostate *25*:132-140.
29. **Chadwick, D.J., T. Kemple, J.P. Astley, A.G. MacIver, D.A. Gillatt, P. Abrams, and J.C. Gingell.** 1991. Pilot study of screening for prostate cancer in general practice. Lancet *338*:613-616.
30. **Deliveliotis, C., G. Alivizatos, A. Karayiannis, D. Kontothanasis, K. Makrychoritis, P. Lysiotis, and M.A. Dimopoulos.** 1995. The value of prostatic

specific antigen in the early diagnosis of prostatic cancer: a Greek view. Br. J. Urol. *75*:637-641.
31. **Jongeneelen, F.J.** 2001. Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. Ann. Occup. Hyg. *45*:3-13.
32. **Nan, H.M., H. Kim, H.S. Lim, J.K. Choi, T. Kawamoto, J.W. Kang, C.H. Lee, Y.D. Kim, et al.** 2001. Effects of occupation, lifestyle and genetic polymorphisms of CYP1A1, CYP2E1, GSTM1 and GSTT1 on urinary 1-hydroxypyrene and 2-naphthol concentrations. Carcinogenesis *22*:787-793.
33. **Wild, C.P. and P.C. Turner.** 2001. Exposure biomarkers in chemoprevention studies of liver cancer. IARC Sci. Publ. *154*:215-222.
34. **Sorsa, M., J. Wilbourn, and H. Vainio.** 1992. Human cytogenetic damage as a predictor of cancer risk. IARC Sci. Publ. *116*:543-554.
35. **Lerman, C. and A.E. Shields.** 2004. Genetic testing for cancer susceptibility: the promise and the pitfalls. Nat. Rev. Cancer *4*:235-241.
36. **Aretz, S., S. Uhlhaas, Y. Sun, C. Pagenstecher, E. Mangold, R. Caspari, G. Moslein, K. Schulmann, et al.** 2004. Familial adenomatous polyposis: aberrant splicing due to missense or silent mutations in the APC gene. Hum. Mutat. *24*:370-380.
37. **Kennedy, R.D., J.E. Quinn, P.B. Mullan, P.G. Johnston, and D.P. Harkin.** 2004. The Role of BRCA1 in the Cellular Response to Chemotherapy. J. Natl. Cancer Inst. *96*:1659-1668.
38. **Rogozinska-Szczepka, J., B. Utracka-Hutka, E. Grzybowska, B. Maka, E. Nowicka, A. Smok-Ragankiewicz, H. Zientek, J. Steffen, et al.** 2004. BRCA1 and BRCA2 mutations as prognostic factors in bilateral breast cancer patients. Ann. Oncol. *15*:1373-1376.
39. **Nicoletto, M.O., M. Donach, A. De Nicolo, G. Artioli, G. Banna, and S. Monfardini.** 2001. BRCA-1 and BRCA-2 mutations as prognostic factors in clinical practice and genetic counselling. Cancer Treat Rev. *27*:295-304.
40. **Bilous, M., C. Ades, J. Armes, J. Bishop, R. Brown, B. Cooke, M. Cummings, G. Farshid, et al.** 2003. Predicting the HER2 status of breast cancer from basic histopathology data: an analysis of 1500 breast cancers as part of the HER2000 International Study. Breast *12*:92-98.
41. **Goldknopf, I.L., H.R. Park, and H.M. Kuerer.** 2003. Merging diagnostics with therapeutics through proteomics. IVD Technology:39-40.
42. **Attard, T.M., C. Cuffari, T. Tajouri, J.A. Stoner, M.T. Eisenberg, J.H. Yardley, S.C. Abraham, D. Perry, et al.** 2004. Multicenter experience with upper gastrointestinal polyps in pediatric patients with familial adenomatous polyposis. Am. J. Gastroenterol. *99*:681-686.
43. **Burt, R.W.** 1996. Familial risk and colorectal cancer. Gastroenterol Clin. North Am. *25*:793-803.
44. **Falzetti, D., J.R. Vermeesch, T.L. Hood, E.P. Nacheva, C. Matteucci, M.F. Martelli, H. Van den Berghe, P. Marynen, et al.** 1999. Identification of multiple copies of a 20q-chromosome in a case of myelodysplastic syndrome: a FISH study. Leuk. Res. *23*:407-413.
45. **Correa, P.** 2003. Bacterial infections as a cause of cancer. J. Natl. Cancer Inst. *95*:E3.
46. **Church, J.M., E. McGannon, C. Burke, and B. Clark.** 2002. Teenagers with familial adenomatous polyposis: what is their risk for colorectal cancer? Dis. Colon Rectum *45*:887-889.
47. **Agaoglu, F.Y., Y. Dizdar, O. Dogan, C. Alatli, I. Ayan, N. Savci, S. Tas, N. Dalay, et al.** 2004. P53 overexpression in nasopharyngeal carcinoma. In Vivo *18*:555-560.
48. **Roesch, A., B. Becker, S. Meyer, C. Hafner, P.J. Wild, M. Landthaler, and T. Vogt.** 2004. Overexpression and hyperphosphorylation of retinoblastoma protein in the progression of malignant melanoma. Mod. Pathol. Oct. 22; [Epub ahead of print].
49. **Okano, H., H. Shinohara, A. Miyamoto, K. Takaori, and N. Tanigawa.** 2004. Concomitant overexpression of cyclooxygenase-2 in HER-2-positive on Smad4-reduced human gastric carcinomas is associated with a poor patient outcome. Clin. Cancer Res. *10*:6938-6945.
50. **David, O., J. Jett, H. LeBeau, G. Dy, J. Hughes, M. Friedman, and A.R. Brody.** 2004. Phospho-Akt overexpression in non-small cell lung cancer confers significant stage-independent survival disadvantage. Clin. Cancer Res. *10*:6865-6871.
51. **Zhou, X., M. Tan, V. Stone Hawthorne, K.S. Klos, K.H. Lan, Y. Yang, W. Yang, T.L. Smith, et al.** 2004. Activation of the Akt/mammalian target of rapamycin/4E-BP1 pathway by ErbB2 overexpression predicts tumor progression in breast cancers. Clin. Cancer Res. *10*:6779-6788.
52. **Stamey, T.A., N. Yang, A.R. Hay, J.E. McNeal, F.S. Freiha, and E. Redwine.** 1987. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N. Engl. J. Med. *317*:909-916.
53. **Devine, P.L., M.A. McGuckin, and B.G. Ward.** 1992. Circulating mucins as tumor markers in ovarian cancer. Anticancer Res. *12*:709-717.
54. **Eisinger, F., J. Jacquemier, C. Charpin, D. Stoppa-Lyonnet, B. Bressac-de Paillerets, J.P. Peyrat, M. Longy, J.M. Guinebretiere, et al.** 1998. Mutations at BRCA1: the medullary breast carcinoma revisited. Cancer Res. *58*:1588-1592.
55. **Fisher, C.J., M.K. Egan, P. Smith, K. Wicks, R.R. Millis, and I.S. Fentiman.** 1997. Histopathology of breast cancer in relation to age. Br. J. Cancer *75*:593-596.
56. **Schrag, D., K.M. Kuntz, J.E. Garber, and J.C. Weeks.** 2000. Life expectancy gains from cancer prevention strategies for women with breast cancer and BRCA1 or BRCA2 mutations. JAMA *283*:617-624.
57. **Paez, J.G., P.A. Janne, J.C. Lee, S. Tracy, H. Greulich, S. Gabriel, P. Herman, F.J. Kaye, et al.** 2004. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science *304*:1497-1500.
58. **Ross, J. and J. Fletcher.** 1998. The HER-2/neu oncogene in breast cancer: prognostic factor, predictive. Stem Cells *16*:413-428.
59. **Dusinska, M., A. Collins, A. Kazimirova, M. Barancokova, V. Harrington, K. Volkovova, M. Staruchova, A. Horska, et al.** 2004. Genotoxic effects of asbestos in humans. Mutat. Res. *553*:91-102.
60. **Santin, A.D., F. Zhan, S. Bellone, M. Palmieri, S. Cane, E. Bignotti, S. Anfossi, M. Gokden, et al.** 2004. Gene expression profiles in primary ovarian serous papillary tumors and normal ovarian epithelium: identification of candidate molecular markers for ovarian cancer diagnosis and therapy. Int. J. Cancer *112*:14-25.
61. **Lleonart, M.E., S. Ramon y Cajal, J.D. Groopman, and M.D. Friesen.** 2004. Sensitive and specific detection of K-ras mutations in colon tumors by short oligonucleotide mass analysis. Nucleic Acids Res. *32*:e53.
62. **Lu, C., J.C. Soria, X. Tang, X.C. Xu, L. Wang, L. Mao, R. Lotan, B. Kemp, et al.** 2004. Prognostic factors in resected stage I non-small-cell lung cancer: a multivariate analysis of six molecular markers. J. Clin. Oncol. *22*:4575-4583.
63. **Ross, J.S., J.A. Fletcher, K.J. Bloom, G.P. Linette, J. Stec, W.F. Symmans, L. Pusztai, and G.N. Hortobagyi.** 2004. Targeted therapy in breast cancer: the HER-2/neu gene and protein. Mol. Cell Proteomics *3*:379-398.
64. **Mao, G.E., G. Morris, Q.Y. Lu, W. Cao, V.E. Reuter, C. Cordon-Cardo, G. Dalbagni, H.I. Scher, et al.** 2004. Glutathione S-transferase P1 Ile105Val polymorphism, cigarette smoking and prostate cancer. Cancer Detect. Prev. *28*:368-374.
65. **Yang, G., X.O. Shu, Z.X. Ruan, Q.Y. Cai, F. Jin, Y.T. Gao, and W. Zheng.** 2004. Genetic polymorphisms in glutathione-S-transferase genes (GSTM1, GSTT1, GSTP1) and survival after chemotherapy for invasive breast carcinoma. Cancer.
66. **Keshava, C., E.C. McCanlies and A. Weston.** 2004. CYP3A4 polymorphisms—potential risk factors for breast and prostate cancer: a HuGE review. Am. J. Epidemiol. *160*:825-841.
67. **Berger, A.C., I.M. Meszoely, E.A. Ross, J.C. Watson, and J.P. Hoffman.** 2004. Undetectable preoperative levels of serum CA 19-9 correlate with improved survival for patients with resectable pancreatic adenocarcinoma. Ann. Surg. Oncol. *11*:644-649.
68. **Nonaka, M.M., D.M. Kataoka, S.M. Yamamoto, A.M. Bito, J.M. Matsuoka, T.M. Kawada and T.M. Takaba.** 2004. Pre- and post-operative serum carcinoembryonic antigen in primary lung adenocarcinoma. Ann. Thorac. Cardiovasc. Surg. *10*:281-284.
69. **Bendardaf, R., H. Lamlum, and S. Pyrhonen.** 2004. Prognostic and predictive molecular markers in colorectal carcinoma. Anticancer Res. *24*:2519-2530.
70. **Yoshimasu, T., R. Sasaki, S. Oura, I. Hirai, Y. Kokawa, H. Tanino, T. Sakurai, and Y. Okamura.** 2004. A case of serum CEA disappearance curve after resection of breast carcinoma. Breast Cancer *11*:203-205.
71. **Xing, P.X., G.P. Young, D. Ho, M.A. Sinatra, P.B. Hoj, and I.F. McKenzie.** 1996. A new approach to fecal occult blood testing based on the detection of haptoglobin. Cancer *78*:48-56.
72. **Bresalier, R.S., J.C. Byrd, D. Tessler, J. Lebel, J. Koomen, D. Hawke, E. Half, K.F. Liu, et al.** 2004. A circulating ligand for galectin-3 is a haptoglobin-related glycoprotein elevated in individuals with colon cancer. Gastroenterology *127*:741-748.
73. **Meky, F.A., P.C. Turner, A.E. Ashcroft, J.D. Miller, Y.L. Qiao, M.J. Roth, and C.P. Wild.** 2003. Development of a urinary biomarker of human exposure to deoxynivalenol. Food Chem. Toxicol. *41*:265-273.
74. **Sutphen, R., Y. Xu, G.D. Wilbanks, J. Fiorica, E.C. Grendys, Jr., J.P. LaPolla, H. Arango, M.S. Hoffman, et al.** 2004. Lysophospholipids are potential biomarkers of ovarian cancer. Cancer Epidemiol. Biomarkers Prev. *13*:1185-1191.
75. **Odunsi, K., R.M. Wollman, C.B. Ambrosone, A. Hutson, S.E. McCann, J. Tammela, J.P. Geisler, G. Miller, et al.** 2005. Detection of epithelial ovarian cancer using (1)H-NMR-based metabonomics. Int. J. Cancer *113*:782-788.
76. **Lee, W.N., P. Guo, S. Lim, S. Bassilian, S.T. Lee, J. Boren, M. Cascante, V.L. Go, et al.** 2004. Metabolic sensitivity of pancreatic tumour cell apoptosis to glycogen phosphorylase inhibitor treatment. Br. J. Cancer *91*:2094-2100.
77. **Howe, F.A., S.J. Barton, S.A. Cudlip, M. Stubbs, D.E. Saunders, M. Murphy, P. Wilkins, K.S. Opstad, et al.** 2003. Metabolic profiles of human brain tumors using quantitative in vivo 1H magnetic resonance spectroscopy. Magn. Reson. Med. *49*:223-232.

Address correspondence to:

Sudhir Srivastava
Chief, Cancer Biomarkers Research Group
Division of Cancer Prevention
National Cancer Institute
National Institutes of Health
Executive Plaza North, Room 3142
6130 Executive Boulevard
MSC 7346
Bethesda, MD 20892-7346, USA
e-mail: ss1a@nih.gov