Cancer Biomarkers: Easier Said Than Done

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Background: Biological and technical advances have led to greatly increased research and development of cancer biomarkers. This overview lists some of the challenges and barriers to developing novel effective cancer biomarkers and enablers to facilitate cancer biomarker development.

Methods: Current scientific literature regarding development of biomarkers for cancer and other diseases was reviewed.

Results: Challenges to developing cancer biomarkers include better understanding of biological heterogeneity, including host/tumor heterogeneity; analytical factors, such as interferences and analytical sensitivity; clinical pathologic factors, such as current histopathologic standards; and health service and market factors. More standardized biomarker definitions, standardization of cancer biology terminology, and high-quality reference materials (specimen and clinical data repositories) were identified as factors required to support advances in cancer biomarkers.

Conclusions: With the above enablers, novel cancer biomarkers may be useful, both for assessing early and established neoplasia more precisely and for contributing to the development of novel practical concepts regarding cancer biology.

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Cancer biomarkers are firmly embedded clinically as essential diagnostic modalities to assess cancer. Human chorionic gonadotropin and α-fetoprotein for germ cell tumors, monoclonal serum and urine electrophoretic peaks for myeloma, and prostate-specific antigen for prostate cancer represent only a few of the successful cancer biomarkers now used clinically.

The explosion of research activity in cancer biomarkers over the past decade can be ascribed to the convergence of science, technology, and regulatory and social factors as follows:

- The success and utility of biomarkers such as human chorionic gonadotropin, monoclonal immunoglobulin peaks, and prostate-specific antigen have assisted the detection and assessment of cancer.
- Scientific advances in understanding cell and molecular biology, including the emerging science of genomics and proteomics, have generated a plethora of candidate cancer biomarkers.
- Advances in analytical assay technology have made possible simultaneous multiple (and even multitudinous) assays. Advances in bioinformatics permit large masses of apparently unrelated data to be mined for correlations (1–5). A notable current example using proteomics is the application of surface-enhanced laser desorption/ionization (SELDI) mass spectroscopy of low-molecular-weight plasma peptides to segregate ovarian tumor from nontumor populations (6). Using the most sophisticated techniques and bioinformatics currently available, that study describes patterns that discriminate tumor from nontumor patients, independent of insight as to what aspect of disease the patterns represent. Given the peptide concentrations studied, it is possible that the patterns represent acute-phase reactants or other systemic features associated with disease generally, but not ovarian tumors specifically. It also remains to be determined whether these patterns can detect tumors in asymptomatic patients who are at risk for ovarian cancer. Exciting as these findings appear, there are many steps required for validation of this and other “high tech on spec” approaches to cancer biomarker technology.
- Regulatory trends worldwide toward standard objective means to compare the therapeutic efficacy of new agents have generated the need not only to measure the efficacy of biomarkers, but have also created needs to have similar markers for early detection, disease natural history, and disease activity (7, 8). The requirement for biomarkers extends beyond cancer to many other disease states, particularly those diseases that progress over relatively long time periods (9, 10).
- Social and market forces have questioned the cost and
relative failure of cancer treatment strategies directed toward advanced disease. This has generated an increase in private and public funding directed toward biomarkers for early cancer detection (11). This trend has been supported by the aging, relatively wealthy post-World War II North American population, who believe that wellness and longevity can be achieved, in part, by extensive screening against the presence of early disease.

- Accompanying this activity, there is a major shift in investigative strategy from an orderly inquiry into biological mechanisms toward a “brute force” approach that can be characterized as “collect the set, generate and mine data”. Formerly, in an often incidental manner, a substance would be discovered associated with a tumor or class of tumors. Subsequently, a hypothesis would be generated, tested, and if promising, lead to further investigation, which after several innovations would enlarge our knowledge of tumor biology.

- Perhaps the earliest example of a cancer biomarker is urinary “Bence Jones protein” and the γ-globulin peak associated with plasma cell myeloma. Subsequent study has determined that the peak is, in fact, a monoclonal immunoglobulin or a fraction thereof (typically κ or λ light chains). Specific classes of immunoglobulins or immunoglobulin fragments are associated with specific pathophysiologies. Furthermore, under some circumstances, benign monoclonal peaks without plasma cell neoplasia exist.

- The current investigative strategy fashion, stimulated by the technical success of the genome project, is to accumulate vast libraries of serum, tissues, and DNA with limited information about clinical history, disease state, or the location of tumor samples. These specimens are then subjected to the current array assay technology, producing “profiles” of analytes, some of which, after application of bioinformatic techniques, may correlate with tumor status. That “association is not necessarily causation” is often not considered well. In the best-case scenario, this “boiling the ocean” approach can be construed as data looking for questions.

Just as sorting out the genome provided a large data set to address scientific questions but by itself did little to add to biological insight, we can expect little more from biochemical, molecular, or image analysis profiling by themselves.

The challenges to development of useful cancer biomarkers must be recognized. These include:

- Biological factors
  - Biological heterogeneity

  Like other organisms, among humans there is extensive heterogeneity of biological expression. In neoplasia, progressive biological heterogeneity with transient characteristics of expression is a characteristic of tumor cells (12). Biological heterogeneity is present both among cells within a tumor at a given time point and in cells during the development of tumors from earlier to later time points. This heterogeneity may be affected by radiotherapy or chemotherapy and, presumably, by endogenous host factors.

  Although almost any cancer can be used as an example, the presence and concentrations of estrogen and progesterone receptors in breast cancer are notable. Of interest, as tumors become more malignant, the phenotypic characteristics of tissue origin tend to diminish with corresponding changes in the marker. For example, the benign neural tumor, neurilemoma, expresses 5-100 as a specific marker, whereas neurofibrosarcomas, which sometimes grow out of these benign tumors, do not.

  Biological heterogeneity includes multiple metabolic pathways to the same endpoints and the variable metabolism of biomarkers, including posttranslational transmodifications. Some or all of these factors may affect the detection and concentrations of the analytes.

- Variation with age, disease, and benign neoplasia

  Normal, reparative, aging, or other physiologic or pathologic processes may generate biomarker profiles similar to that in malignancy. “Cancer” biomarkers may also be present in benign neoplastic disease, which careful longitudinal clinical study has shown does not proceed to malignancy (13, 14). A vitally important and humbling example is the demonstration that oncogene markers such as c-erbB-2, p53, and cyclin D1, commonly thought to be cancer biomarkers, are also present in patients with benign breast disease who have been followed clinically for 15 years or longer without neoplastic progression.

- Exogenous substances that affect biomarker presence and concentration

  Foods, drugs, and natural alternative therapies are “interferences” well known to clinical biochemists. Well known are the many medications and foodstuffs that interfere with vanillylmandelic acid or 5-hydroxyindole acid, markers of pheochromocytoma and carcinoid tumors, respectively (15). These examples render a cautionary note for gene expression profiles. Undoubtedly, most expression profiles will be imitated by benign conditions or, alternatively, enhanced/suppressed by medications for concurrent conditions.

- Clinical pathologic factors

  This requires defining more precisely and standardizing concepts of the biological events against which biomarkers are to be measured. This difficult task includes defining normal variation, preneoplastic states, and neoplasia grade and staging with sufficient precision to make cancer biomarkers useful. For preneoplasia that is microscopically evident and for overt tumors, there is a long history of medical art and science...
that distinguishes classes of tumors with different prognosis, e.g., a 50% five-year survival, based on morphology and, increasingly, immunohistochemical and molecular diagnostic techniques. There is considerable need to refine the prognosis for individual cancer patients who share tumor class as defined by current techniques. Of particular interest, preneoplastic states and states of early tumor recurrence need to be detected for which there is no tissue visible by imaging and for which no systemic markers are currently available. These needs can be judged best by past examples. Radiologic calcification as a marker of breast tumors and apoptosis or lack of apoptosis as tumor features are now well known. These marker features have always been present but, formerly, were not recognized. Furthermore, as these markers became accepted, specialized techniques for easier detection were developed.

- Analytical sensitivity and detection limit

The advances in sensitivity of analytical technology are such that it is often possible to detect a cancer biomarker, whether it be a biochemical analyte, DNA, or circulating cancer cells. Detection limits may be sufficiently low to allow detection below concentrations that have biological importance. The biomarker may detect, appropriately, an important phenomenon, but endogenous regulatory mechanisms (not measured) may maintain the neoplastic process under firm control. The implications are that clinical detection and measurement of biomarkers of this type, at worst, could lead to unnecessary investigation and therapy or, at best, unnecessary chronic anxiety for the patient.

- Health service factors

It is not sufficient for a cancer biomarker to detect a particular phase of neoplasia. To be successful, the biomarker must also fit within the profile of health service factors with respect to cost-effectiveness, cost benefit, and the relative value of the cancer biomarker strategy for cancer burden reduction (16).

Given the above variables, it is indeed remarkable that any useful cancer biomarkers exist (17, 18). Even after more than 150 years of cell science, it must be recognized that our conceptual framework of cancer biology remains inadequate to recognize the ideal or optimal biomarker for most cancers. Furthermore, even if, as expected, our perspectives will change over time, we need to understand what we are looking for before investments in the search and evaluation for cancer biomarkers will be effective.

Given the current reality, in the near future cancer biomarker investigators will generate and be confronted with analytes and data that are orders of magnitude greater than were available even 1 year ago. Considering these theoretic and practical difficulties, what advances will facilitate the development of cancer biomarkers? A short list includes:

- Defining the biology of each cancer and the biology of the cancer process with precision

This involves not only defining what is known, but also attempting to define or circumscribe what is unknown. This requires enhanced interactions among investigators of different disciplines, including clinical biochemists, pathologists, and cell and molecular biologists. It requires not just defending current tumor assessment methodology or the cancer biomarker assay du jour, but challenging assumptions and the rigor of studies.

An urgently needed skill set includes a greater appreciation of the biokinetics of both cancers and cancer biomarkers. These skills, analogous to pharmacokinetics, will show how biomarkers change in presence or concentration, thereby permitting more dynamic views of how cancers evolve.

- Defining host biology: pharmacogenomics and pharmacoproteomics

Biological profiling does have the prospect of individualizing pharmacotherapy, maximizing efficacy, and minimizing toxicity (19). It is likely that biomarkers that simultaneously reflect both cancer activity and individual sensitivity to therapy will be developed.

- Defining biomarkers and surrogate endpoints

Although concepts of biomarkers have been discussed for decades (20), research and regulatory agencies have recently taken an increased interest in developing consensus about definitions (7, 8). Biomarkers have been defined from various viewpoints. For example, one classification defines type 0 as a natural history marker, type 1 as biological activity markers, and type 2 as surrogate markers for clinical efficacy (21). Other investigators looking at epidemiologic aspects reach back even earlier in the neoplastic process, searching for biomarkers of exposure to carcinogens, familial genetic predisposition, and individual sensitivity to cancer (22). Because cancer and nonneoplastic diseases may be positively or negatively related, it is extremely important that the biomarker definitions that emerge from consensus can be applied in a similar manner not only to cancer, but also to other disease processes.

- Standardization and stringency of analytical technology

Extensive effort has been made to standardize preanalytical, analytical, and postanalytical methodology for cancer biomarkers (23, 24). Standardization of biomarker assay technology involves considerations beyond analytical sensitivity and specificity. For example, in advancing toward standardized technology, the advantages of comparability between various studies must be weighed against the desire and need for...
innovation and conditions that require protocol flexibil-
ity.

- High-quality specimen and clinical data repositories

The need for such repositories is unquestioned. Vi-
tually every center is rushing to provide resources for
these purposes. Specimen and data repositories repre-
sent major undertakings in which issues such as bioeth-
ics, patient consent, confidentiality, specimen prove-
enance, technical preparation, and storage must be
resolved. Many medical laboratories have extensive
experience with such repositories in the form of tissue
blocks, slides, and pathology reports. Within the guide-
lines prepared by various regulatory agencies, these
laboratories are in the best position professionally to
manage these resources, balancing the interests of the
patient and the research community.

Effective intervention for cancer prevention and early
cancer detection does require more effective cancer
biomarkers. The key enablers for effective cancer markers
call into a critical approach to understanding tumor and
host biology, consensus definitions of biomarkers and
surrogate endpoints, stringent analytical criteria for assay
technology, high-quality specimen and data repositories,
and the management system for these resources. Ad-

dvances in developing effective cancer biomarkers will be
highly dependent on the orderly development of both the
intellectual framework and the extensive investigative
infrastructure among cancer investigators of all disci-
plines worldwide.

New cancer biomarkers are likely to come from in-
sights derived both from analytical interpretation of ar-
rays and molecular profiles as well as from more struc-
tured specific hypothesis-driven biological investigation.
Cancer markers will continue to be developed to assess
neoplasia that is currently recognized as present micro-
scopically or systemically. Of even greater importance,
discovery of novel cancer biomarkers to detect and assess
cancer where there is no other means of detection can be
expected. These markers will, in the first instance, extend
our knowledge of specific tumors within the framework
of cell biology that has been developed over the past 150
years. However, particularly with the use of physical
techniques, e.g., spectroscopy of various types, the poten-
tial exists to develop powerful cancer biomarkers based
on truly novel concepts of cell and cancer biology.

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