Review of Circulating Tumor Markers: From Enzyme, Carcinoembryonic Protein to Oncogene and Suppressor Gene*

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ABSTRACT

The ease and non-invasive nature of the procedure for drawing blood has made it possible to measure circulating tumor markers for cancer screening, diagnosis, follow-up and early detection of recurrence. Even though a tumor-specific marker has not yet been found, the specificity and sensitivity of currently used tumor markers have improved over the last several decades as they have progressed from enzyme, hormone and carcinoembryonic protein to monoclonal antibody-defined epitope and finally, in recent years, to oncogene, suppressor gene and their encoded protein product. Both phenotype and genotype are included in these latest new tumor markers. Unlike earlier tumor markers, they can be identified with specific biological processes regulating cell growth, such as the cell cycle, angiogenesis, apoptosis and cell adhesion. Any elevation of these new tumor markers, therefore, can be used to identify defects in a specific metabolic pathway and facilitate the design of effective drug therapy.

Introduction

For the last several decades, considerable effort has been directed to searching for cancer markers that would enable the early diagnosis and screening of cancer, monitoring treatment and early detection of recurrence. These exertions bore fruit with the discovery of circulating tumor markers. The ease and feasibility of frequent blood drawing and urine sampling allowed for development of circulating tumor markers for the diagnosis and management of cancer patients. Bence-Jones protein was the first circulating tumor marker identified that led us to believe that the concentration of some serum or urine proteins might reflect tumor activity and tumor mass and that their quantification might result in a useful determination of the success of therapy. Subsequently, many substances and molecules appearing in circulating blood and urine, such as serum enzymes, serum proteins, many metabolites and hormones, were also employed as tumor marker. As we used more and more serum molecules for the screening and diagnosis of cancer and for the management of cancer patients, we began to refine our knowledge of the qualities of an ideal tumor marker and to understand why the specificity and sensitivity of a tumor marker were important in determining its clinical
value. Although we did not discover a tumor-specific antigen or tumor-specific epitope, guided by these criteria, we were able to develop many new tumor marker tests over the years and thus greatly improve the clinical utilities of tumor markers.

**ENZYMES, SERUM PROTEINS & HORMONES**

During the 1960s, many serum proteins, hormones and enzymes served as tumor markers. Among them, enzymes were most often used. Some of the more frequently employed enzymes at that time are listed in Table I. In the early days, tumor markers were measured by determining enzymatic activity and by electrophoresis, both relatively low sensitivity tests. Most tumor markers were found to lack both the desired specificity and sensitivity, even though their levels reflected tumor progression and paralleled the patient's clinical status. Malignant diseases were detected through the measurement of isoenzymes with different tissue specificities, such as lactate dehydrogenase (LD), creatine phosphokinase (CK) and alkaline phosphatase (ALP). The measurement of specific isoenzymes, instead of the total enzymatic activity, slightly improved the specificity of the enzyme test.

**CARCINOEMBRYONIC PROTEINS**

In the late 1970s, the discovery of carcinoembryonic antigen (CEA) generated a great deal of excitement and initially led us to believe that a tumor-specific antigen had been found. The discovery of CEA prompted an intensive search for more so-called fetal tumor antigens, or carcinoembryonic proteins, that could be used as tumor markers. These carcinoembryonic proteins appeared to be tumor-specific because their synthesis during fetal stage, which was turned off during normal developmental processes, become reactivated in tumor cells. Even though the specificity of CEA did not in the end meet expectations, studies on CEA and other carcinoembryonic proteins provided much valuable information concerning the various properties of circulating tumor markers. For example, it was learned at that time that these tumor markers existed in blood circulation at such low concentrations that the sensitivity of an immunoassay was required for their quantification. Moreover, studies of the blood level of carcinoembryonic protein in malignant diseases led to greater insight into prognosis. It was found that tumor metastases were associated with high blood levels of circulating tumor markers and that much more aggressive therapies would be required to treat patients with poor prognoses.

**ASSOCIATION WITH METASTASES**

A better understanding of the importance of ascertaining the prognosis for cancer patients

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Associated Malignant Disease</th>
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<tbody>
<tr>
<td>Prostatic acid phosphatase</td>
<td>Prostate carcinoma at late stage.</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Acute leukemia; malignant lymphoma; germ cell tumors; metastatic colon, breast and lung</td>
</tr>
<tr>
<td>5′-Nucleotide phosphodiesterase</td>
<td>Lung cancer, liver metastases.</td>
</tr>
<tr>
<td>Sialyltransferase</td>
<td>Nonspecific.</td>
</tr>
<tr>
<td>Fucosyltransferase</td>
<td>Multiple malignant tumors.</td>
</tr>
<tr>
<td>Thymidine kinase</td>
<td>Hodgkins lymphoma, certain leukemias, and small cell carcinoma of the lung</td>
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so that they may receive proper treatment resulted in attempts to identify tumor markers associated with various stages of metastasis as prognostic marker. Because metastasis is linked with an unfavorable outcome, detecting elevated levels of markers associated with metastasis generally means poor prognosis. Among all markers identified with the various stages of metastasis, proteases are the most useful clinical markers: tumor invasion and metastasis were found to be related to elevated levels of proteases.\textsuperscript{7,8} Proteases appear to assist active tumor cells in the penetration of basement membranes and interstitial stroma during the transition from \textit{in situ} to invasive carcinoma.

**Monoclonal Defined Tumor Markers**

Since many tumor markers, such as carcinoembryonic antigens, express multiple epitopes and many of these epitopes are shared by different tumor markers, the nonspecificity of these tumor markers was deemed a result of the use of polyclonal antibodies. As soon as hybridoma technology became available, efforts were invested in replacing the polyclonal with monoclonal antibodies in the tumor marker kit for the purpose of improving assay specificity.\textsuperscript{9} The monoclonal antibody approach might lead us to the identification of tumor-specific markers (or tumor-specific epitopes). Although none of the epitopes identified turned out to be tumor-specific, nonetheless the overall specificity and sensitivity have been greatly improved with the monoclonal kits. In recent years, several monoclonal tumor marker kits have become available commercially (table II). These new monoclonal antibody tumor markers have been the most popular tumor markers used clinically for managing patients with carcinomas.

**Mucin and Blood Group Substances**

It is important to note that the new tumor markers identified by the monoclonal antibodies turned out to be a group of epitopes related to mucins-glycoproteins of high molecular weight molecules.\textsuperscript{10,11} In fact, many of these monoclonal antibody defined epitopes, such as the oligosaccharide portion of mucin glycoproteins, are blood groups. Altered blood group antigens (or their carbohydrate epitopes) are known to be frequently overexpressed in malignant tissues. The overexpression of altered blood group antigens is usually related to the process of tumor progression and is associated with poor prognosis.\textsuperscript{12} It is well known that neoplastic cells may “lose” or “acquire” new antigens that are expressed or not expressed, respectively, by their normal counterparts. Studies also showed that many of these malignancy associated blood group antigens are related to blood groups A, B, H and Lewis antigens and their precursors.\textsuperscript{13,14,15} Incomplete biosynthesis of the major blood group antigens occurs frequently in tumor cells, resulting in the accumulation of precursor-like substances in the tumors. Loss of antigen expression has been found in bladder cancer correlating with invasiveness. Conceivably, monoclonal antibodies against these altered carbohydrate epitopes will prove useful for their identification and for the establishment of tumor marker immunoassays.

**Multiple Markers**

Since most tumor markers, whether polyclonal or monoclonal antibody defined, lack the desired sensitivity and specificity in their clinical applications, it appeared that these areas could be improved with the use of mul-

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**TABLE II**

<table>
<thead>
<tr>
<th>Monoclonal Kit</th>
<th>Associated Major Malignant Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 125</td>
<td>Ovarian carcinoma</td>
</tr>
<tr>
<td>Hybri-BREScan (CA 549) or CA 15–3</td>
<td>Breast carcinoma</td>
</tr>
<tr>
<td>Hybri-CMark (CA 195) or CA 19–9</td>
<td>Pancreatic carcinoma</td>
</tr>
<tr>
<td>CA 72–4</td>
<td>Gastric carcinoma</td>
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</tbody>
</table>
Multiple markers. Multiple markers are usually detectable in both individual serum and plasma specimens. Studies have indicated that use of more than one marker enhances the sensitivity of tumor detection. Also, examination of the pattern displayed by the combination of more than one marker reveals repeatedly that specific patterns appear to associate with different types of tumors.16,17

Emerging New Tumor Markers

Recent studies on growth regulation, the signal transduction pathway and various biological processes involved in controlling cell growth resulted in identification of many new tumor markers. Cancer is now recognized as essentially a disorder of growth regulation. Any biological process associated with growth control can have a potential impact on tumorigenesis. Only in recent years has there been a better understanding of how the signal transduction pathway regulates cell growth and how it interacts with many individual biological processes to impact cell proliferation.18 We now understand not only how the cell cycle is regulated and how the process of "apoptosis," or programmed cell death, regulates cell growth, but also how the adhesion of a cell to other cells and to its microenvironment impacts cell proliferation. We learned recently why many growth factors, receptors and components of signal transduction, apoptosis, cell cycle and angiogenesis are either oncoproteins or suppressor proteins. Conceivably, these proteins, which are connected through the signal transduction pathway and associated with important biological processes of cell growth, are potential tumor markers and prognostic factors. The major difference between the new emerging tumor markers and those once employed is the fact that the former are known to be associated with a particular biological process. The concentration of these new tumor markers has an effect on individual metabolic reactions and an impact on overall cell growth. Consequently, understanding the relationship of a specific defect and tumorigenesis will facilitate a definitive design of drugs for therapy.

1 Oncogene, suppressor gene and their encoded proteins

By definition, an oncogene is a mutant proto-oncogene. The product (phenotype) of the oncogenes affect practically every level of signal transduction and growth-related biological processes which cause cancer cells to lose the regulatory constraints of their activity19 and continue to promote cell proliferation without external activation signals. Oncogenes and their oncoproteins are useful tumor markers especially for predicting prognoses and identifying people at risk (table III). For example, detection of c-erbB-2 gene amplification or protein overexpression has been shown to correlate with poor short-term prognosis or an elevation in the increased risk of tumor recurrence. Detection of c-erbB-2 overexpression appears to be an event associated with the early stages of breast cancer.20,21

On the other hand, the prime function of suppressor genes is to stop cell growth. The loss or inactivation of these genes creates inactive gene products, resulting in failure to halt cell growth and eventual development of a wide variety of human cancers. Therefore, measurement of suppressor genes (either their absence or mutation) is also useful in determining prognosis. The most popular of all suppressor genes is p53.22 Up to 50 percent of human cancers are found to involve p53 mutations. Many studies have demonstrated that increased expression of mutant p53 correlates with a more rapid relapse and diminished survival in women with node-negative breast cancer.

2 Susceptibility genes

Mutant genes related to cell growth can be inherited. They are most often found in families at high risk for cancer, hence the term "cancer susceptibility genes." The detection of mutant tumor suppressor genes could also signal a poor prognosis. Screening the mutant gene or its protein product can be used to identify families, or individual members in the family, at high risk. BRCA1 and BRCA2 are
two susceptibility genes currently being screened for breast cancer predisposition, even though carriers of BRCA1 mutations are also at increased risk for ovarian, colon and prostate cancer. BRCA2 the second susceptibility gene for breast cancer, is associated with a greater risk of breast cancer in men.

(3) Association with angiogenesis

There are positive and negative angiogenic factors. The quantification of elevated angiogenic factors in the blood and urine of cancer patients is being evaluated as a determinant in prognosis and guide to therapy. To monitor the efficacy of anti-angiogenic therapy, the quantification of several markers has been developed for various locations: in the serum and urine.

(4) Association with cell cycle

Cyclins are proteins which regulate the progression of a cell through the cell cycle. By complexing with cyclin dependent kinases (cdk), they play an important role at different stages of the cell cycle in the regulation of growth of both normal and neoplastic cells. The periodic appearance of the cyclins in distinct phases of the cell cycle suggests that they can be used as markers for the proliferation of tissues. Mutations in the cyclin gene have now been found in a variety of cancers, and many oncogenes and suppressor genes may encode proteins operating throughout the cell cycle, leading eventually to uncontrolled cell growth.

(5) Association with cell adhesion

Soluble adhesion molecules can be found in blood and tissue fluids. Three classes of adhesion molecules are known to exist: selectins, integrins and the immunoglobulin super-family. Appearance of adhesion molecules is considered a sign of the conversion of normal to malignant cells, and changes in molecular expression of major adhesion molecules were found to be related to malignant transformation, tumor spreading and immortalization.

Two major changes have occurred in the selection of tumor markers in recent years. Our improved understanding of detailed biological processes and metabolic pathways associated with growth regulation allows us to identify and select tumor markers with known specific functions and biological roles in controlling cell growth. Another change involves the measurement of gene mutations for risk assessment, prognosis and the identification of
aggressive tumors. Recent attempts have been made to measure both the genotypes and phenotypes of oncogenes and suppressor genes and to identify inherited and sporadic mutations. The recent success of using plasma DNA, not the lymphocyte DNA, to detect sporadic mutant genes made it possible to take DNA samples from blood circulation rather than to rely on tissue biopsies.

References