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BIOCHEMICAL MARKERS FOR CANCER

edited by

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1

IMMUNOLOGICAL APPROACH TO THE BIOCHEMICAL MARKERS FOR CANCER

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I. INTRODUCTION

During the past 15 years there has been a rapid increase in interest and information on the existence and potential clinical value of human tumor-associated antigens. This has led to extensive efforts at immunotherapy and immunodiagnosis of cancer, and at immunological monitoring of cancer patients.

Despite considerable progress in this field, recently there has been a substantial amount of skepticism about these applications, and much of

this can be traced to uncertainties about the very existence of human tumor-associated antigens. Part of this uncertainty can be attributed to problems in semantics. Early workers in tumor immunology looked for and described "tumor-specific antigens," i.e., antigens on tumor cells that were qualitatively different from antigens on any normal cells. The complete specificity required by this definition is quite difficult to prove and, with few exceptions, may not be correct. The main problem is to demonstrate sufficiently that a tumor antigen is entirely absent from normal tissues at any time in development. Many of the currently used techniques are relatively insensitive and could fail to detect the presence of small amounts of antigen. Furthermore, some antigens may occur only in some types of normal cells or in cells at one stage of differentiation.

In general it would seem most satisfactory to examine closely normal cells of the same organ and of the same histological type as the cancer cells. In addition it may frequently be necessary to study precursors of mature differentiated cells, either from embryos or from sites of active regeneration. Several of the tumor antigens which will be discussed below were originally thought to be tumor-specific or present only in tumor cells and embryonic cells. However, more concern for this issue and more sensitive techniques have shown that many antigens are present, at least in low amounts, in some normal cells after birth.

Because of these difficulties in demonstrating that an antigen is tumor-specific, the term "tumor-associated" has become popular. The term "tumor-associated antigen" (TAA) is essentially an operational definition for a substance that can be found on a tumor cell but is undetectable in the cells of a normal adult individual. By this definition an antigen detectable only in embryonic cells and tumor cells, a so-called oncofetal antigen, would be considered tumor-associated. Tumor-associated is often a provisional designation for an antigen that has not been fully characterized, or whose controls for specificity have not been, or for practical reasons cannot be, complete.

Beyond this retreat from tumor-specific to tumor-associated antigens, there is an increasing impression that if one looks hard enough, and with sufficiently sensitive techniques, most TAAs will be found to be present in some normal cells. This may be particularly true for TAAs that are common to a number of tumors of the same type. In animal tumor systems one category of common antigens that do appear to be restricted to tumor cells are those induced by oncogenic viruses. Even some of those may be present on morphologically normal cells infected by the viruses, long before malignant transformation occurs [1,2]. In the absence of a virus association with most human tumors, it is rather difficult to account for common, truly tumor-associated antigens. Of the common human TAAs whose specificities have been very extensively examined, the following have been shown to be the actual distribution of the antigens: (1) antigen with large quantitative differences in expression in tumor cells versus

normal cells; (2) antigen well represented in normal tissues, but only in those of a particular type; (3) antigen expressed only or predominantly in normal cells at a particular stage in differentiation; (4) antigen that is detected in the circulation only of cancer patients, but it is actually a precursor molecule or a degradation product of a normal serum protein. Examples of antigens found in some of these categories will be discussed later.

It should be noted that tumor antigens which are ultimately shown to also be on normal cells may still be quite useful clinically. For example, for immunodiagnostic tests it must be possible to detect some consistent difference between cancer and noncancer. It is desirable but not necessary that the difference be qualitative. Quantitative differences between cancer patients and controls could also be sufficient. It would just be necessary to determine carefully the range of values in normal individuals and in patients without cancer.

To measure or localize TAAs or other immunological markers in cancer patients, most studies have relied on antibodies produced against these antigens in heterologous species. Unless highly purified antigens were used for immunization, the antisera usually reacted against many normal antigens and had to be absorbed extensively to render them specific for tumor-associated antigens. This should not be a problem for immune sera from cancer patients, and one might expect such sera to be more practical reagents for immunodiagnosis. In fact, however, the antitumor reactivity of patients' sera has usually been relatively weak, and the sera have not lent themselves well to development of radioimmunoassays or similar sensitive techniques for quantification of antigen concentrations.

In this chapter I will discuss the types of clinical applications for tests for TAAs and the issues and problems related to each of the possible uses.

II. POTENTIAL CLINICAL APPLICATIONS

Before discussing any of the particular markers, it is important to consider the possible objectives for use of these tests and the issues and problems related to each type of application. Table 1 summarizes some of the major potential applications for these assays. It is important to note at the outset that only a few tests for human TAAs have been definitely shown to have a place in clinical oncology. As will be discussed below, results with a number of assays are quite promising, and one major reason for the lack of detailed clinical information is that it has been difficult to satisfactorily transfer the technology from the research laboratory to the bedside. Many of the problems are not unique to tests for TAAs but are also true for other types of laboratory diagnostic tests, including some that have been available for many years and some that have been incorporated into widespread use without real validation or objective assessment of utility.

TABLE 1 Potential Clinical Applications of Immunological Tests for Tumor Markers

1. Detection: screening of populations and high-risk groups
2. Aid in diagnosis of patients with signs or symptoms suggestive of cancer
3. Aid to histopathological evaluation of Tumors
4. Aid in staging of cancer patients
5. Localization of tumor and detection of metastases
6. Serial monitoring to determine efficacy of therapy and to detect recurrence or metastases

In regard to diagnostic applications of these tests, the following can be listed as general criteria for useful tumor markers:

1. A first, obvious point is that the test needs to be able to detect some consistent qualitative or quantitative difference between cancer and noncancer.

2. A good diagnostic test should have a high degree of specificity; there should be very few false positives, i.e., individuals without cancer who have tests indicating cancer. In this sense the percent specificity of a test may be defined as:

(1–Incidence of false positive tests) × 100,

where

$$\text{Incidence of false positive tests} = \frac{\text{no. positive tests among individuals without cancer}}{\text{total noncancer individuals tested}}$$

3. Also, the test should be very sensitive and have few false negative results, i.e., it should be able to detect cancer in a large proportion of cancer patients. The percent sensitivity of a test may be defined as:

(1–Incidence of false negatives) × 100,

where

$$\text{Incidence of false negatives} = \frac{\text{no. negative tests among cancer patients}}{\text{total cancer patients tested}}$$

A particularly useful test for a tumor marker would be one that was positive even in cancer patients with localized tumors or small, metastatic deposits which were asymptomatic and undetectable by conventional diagnostic tests.

4. Some tests for tumor markers give only qualitative results, i.e., they are either positive or negative. However, a test is much more valuable if it provides quantitative information on the levels of the marker. Tests that have a large quantitative range between clinically detectable tumors and absence of tumors are particularly useful, since they offer the possibility of closely monitoring changes with tumor burden, and since they

are most likely to provide indications of small amounts of tumor. Since TAAs are products of tumor cells themselves, if they are released into the circulation their levels would be expected to depend on the mass of tumor. However, a variety of factors may influence the levels of tumor products:

(a) The number of tumor cells present.
(b) The proportion of tumor cells synthesizing the antigen and the synthetic rate per cell. Only certain cells within a tumor may make the TAA, and production may vary with the phase in the cell cycle and with the stage of differentiation of the cell.
(c) The location of the marker within the tumor cell and the mechanism for release from cells and entry into circulation. Some TAAs are cell membrane constituents or secretory products and may be shed or released from viable cells. Other TAAs may be intracellular constituents which would only be released when the tumor cells lose viability. Some TAAs released from solid tumors might enter the circulation in appreciable quantities only after invasion of blood vessels. With such antigens levels in the region of the tumor or in directly contiguous body fluids or excretions might be much higher than in the circulation, and testing of these might be more useful than tests on serum.
(d) The half-life of the antigen in the circulation can also vary considerably, depending on the size and nature of the substance. If the circulating antigen is immunogenic to the host and antigen-antibody complexes are formed, it is likely to be cleared much more rapidly than would a nonimmunogenic marker. When markers are not tumor products but rather are produced in response to tumor growth, the relationship between levels of response and the mass and extent of spread of the tumor might be quite different. It is not possible to set down any general principles for such reactive markers.

5. It would be very helpful if a test to be used for initial detection or diagnosis could provide information about the tumor type and location. One of the main concerns about detecting occult, clinically undetectable cancer is the difficulty in determining what type of cancer it is and where it is located. Clearly, at present more information than a diagnosis of "cancer, type and site unknown" would be needed for rational therapy. Therefore specificity of the TAA for a particular organ site or histologic type of cancer is an important factor initially, but it is not as essential for monitoring previously diagnosed patients.

A further issue concerns not so much tests for TAAs themselves, but the design of the studies to evaluate the usefulness of the marker. It is essential that the measurements and the data analyses be performed

objectively, without knowledge of the clinical diagnosis or status of the patient. To accomplish this it is important that the laboratory receives coded specimens, without any identifiers as to source or type of donor. Beyond this it is necessary to design appropriate studies for the particular clinical application for which the tumor marker will be used. Adequate studies of this type have been performed with very few of the available tests for TAAs, and even then only for some of the possible clinical applications. The oncologist can and should play a central role in designing good studies, to provide solid information on the clinical value of a test.

Another point to emphasize in regard to the usefulness of various assays for one or more of the clinical applications is that a single test may not have sufficient specificity or sensitivity, but that the simultaneous use of several tests may provide highly discriminatory data. It is possible that assays for two or more immunological or biochemical markers would have additive or synergistic effects for improving the sensitivity or specificity of detection of tumor cells.

A. Screening

The use of tests to screen for cancer is probably the most difficult of the various potential applications to bring to fruition. If a test has been shown objectively to discriminate well between cancer and control groups, it then has to be evaluated for its use in screening by a study with an appropriate design. Despite the large number of tests for TAAs which have been developed, only a few have been directly evaluated for their use in screening. Many factors can affect the feasibility of a particular test for screening purposes. Most of these factors need to be extensively considered before a study on the possible usefulness of screening can be initiated.

1. It is particularly important that the assay be relatively simple and practical for application to testing of very large numbers of specimens or individuals. The procedures must be sufficiently well developed and standardized, so that reproducible results can be obtained over time and in many laboratories.

2. A suitable population must be available for study. For rapid identification of a useful test, it is very helpful to identify populations or families at high risk of developing cancer. It is very important to have sufficient access to the population, to permit retesting as appropriate, and to perform extensive clinical evaluations, particularly of the test-positive individuals. Furthermore, most of the individuals in the population must be available for clinical follow-up over a period of several years to determine which initially disease-free individuals, among both the test-positive and test-negative individuals, subsequently develop cancer.

3. Of fundamental concern is the specificity of the assay. It is difficult to make general statements about the acceptable levels of specificity

for screening tests. However, it would be important for a new screening test to be shown to have better specificity than currently available detection techniques, or to lead to improved specificity when used in conjunction with these procedures.

4. A further important issue is that the test should be very sensitive. To be useful as a screening procedure, the test should be able to detect asymptomatic individuals bearing small, localized tumors at a time when the disease is treatable and has not yet metastasized. The longer the lead time that a test provides (i.e., the interval between test positivity and clinical detection of disease), the more likely it is that the test will contribute to better response to therapy and to survival. It should be noted that, in order to determine accurately the lead time for a particular assay, tests need to be performed repeatedly to establish the point when the test first becomes positive. The amount of lead time a test might provide, and the likely clinical benefits to be accrued by early detection of disease, are likely to be determined considerably by the rate of tumor growth. Screening tests are more likely to be useful for slowly growing tumors with long latent periods than for explosively growing tumors that metastasize early.

B. Aid in Diagnosis of Patients With Signs or Symptoms

Some of the issues discussed above for screening also apply to the application of tests for TAAs as adjuncts to the diagnosis of cancer in patients with signs and/or symptoms suggestive of cancer. However, the concerns regarding the sensitivity and specificity of the assay are somewhat different. In regard to specificity in this setting, one is not concerned with identifying the small proportion of individuals with cancer out of a very large population of normal individuals. Rather it is necessary to discriminate among a group of individuals with disease, to determine which have malignant versus benign diseases. This presents particular problems since most forms of cancer arise in older populations with a variety of underlying benign chronic diseases that often affect the same organ system as the cancer. However, if a major cause of false positive results is a benign condition or conditions not involving the same organ system as the cancer, e.g., the liver, it may be possible to perform the relevant test to rule out those other conditions.

In regard to sensitivity, the test would not have to be able to detect very small, asymptomatic lesions. However, to be useful as a diagnostic adjunct, it should still be capable of detecting most resectable or otherwise treatable, localized tumors.

C. Aid to Histopathological Evaluation of Tumors

Recently the value of evaluating tumor specimens for the presence of various TAAs and markers has begun to be increasingly appreciated. This

analysis may provide assistance in the histopathological classification of the tumor. The presence or the amount of a marker may provide useful prognostic information, since these factors may reflect the state of differentiation, immunogenicity, or metastatic potential of the tumor.

Another very important aspect of marker evaluation in tumors is to provide needed information for the subsequent monitoring of the patient. Identification of one or more markers within the tumor would provide a solid basis for using the assays for those markers to follow the course of disease. Although similar information might be obtained by testing for the markers in a pretherapy serum sample, direct examination of the tumor is likely to be more sensitive and specific.

In patients with small, localized tumors, the serum levels before therapy are often in the normal range despite active production of the markers by the tumor. Therefore the failure of the patient to have an initial elevated marker level should not rule out the possible later use of that marker. In fact, with some of the markers discussed below, such a disparity between tumor and circulatory levels has been noted. In contrast, failure to detect a marker within the tumor would make it much less likely to be subsequently detectable. However, although there is little evidence for this, it remains possible that some primary tumors would be negative for a TAA while the metastases might become positive. This could be envisioned if production of the antigen were more likely in a less differentiated or more aggressively growing tumor cell. This possibility needs to be directly explored.

Very recently, improved methods have been developed for examining markers within tumors. In addition to the usual studies of intact cells or tissue sections by immunofluorescence, it is now possible to look for the distribution of markers in fixed and stained tissue sections, using conventional light microscopy. This has been made possible by the development of immunoperoxidase staining techniques. Using this procedure, one can now accurately determine both the presence of the antigen and its location within various cell types in the tumor.

D. Aid in Staging of Cancer Patients

Tests for TAAs in newly diagnosed cancer patients, before any therapy or after primary surgical removal of tumor, might be very useful as an aid in assessing the stage of disease. Since the circulating levels of some TAAs or the degree of immunologic reactivity has been found to depend on the overall tumor burden and on the extent of spread of the tumor, tests on preoperative specimens can provide useful prognostic information. For example, elevated pretherapy levels of TAAs, particularly when quite high, may suggest the presence of metastases and poor prognosis. The test results might also reflect the state of differentiation of the tumor and its inherent aggressiveness.

Most studies on this aspect of marker utilization have been performed on groups of patients to determine the overall relationship between marker level and extent of tumor, as determined at surgery and by subsequent clinical course. To supplement information obtainable by other means, the marker data should be able to make prognostic discriminations among patients with the same clinical or histopathological stage of disease. If this is possible, then the use of markers in conjunction with clinical and other laboratory information could provide the basis for improved staging of patients and for the administration of therapy appropriate to the assessed extent of disease.

It should be noted that the type of data gathered in this area provides information on the probable prognosis of patients within a group, rather than on the clinical status of the individual patient. As with other staging criteria, the results of a test for TAA could not be taken as definitive evidence for occult metastases versus localized tumor.

One of the major challenges to the field of immunodiagnosis is to make the transition from population studies to a study in which statements can be made about individual patients, and on which therapy or other important clinical decisions may be based with some degree of certainty. One promising approach is the testing for levels of a circulating TAA after primary therapy, especially surgery, to determine whether all of the tumor was removed or eradicated. Persistence of elevated levels might provide strong evidence for residual tumor at the primary site or for regional or distant metastases. For example, after mastectomy for breast cancer, such a finding could indicate the need for axillary lymph node dissection and removal, or for radiotherapy or chemotherapy. On the other hand, the fall of elevated TAA levels into the normal range might provide some assurance of curative surgery, and might obviate the need for further therapeutic maneuvers. As will be discussed below, some of the tests are already being examined for this application.

To base important clinical decisions on marker data, a number of factors need to be considered: The assays for markers should be quantitative, and it would be desirable for them to be very sensitive so that they could reflect changes in levels over a wide range, hopefully 10-fold or more differences in concentration between the initial elevated values and the normal levels.

It must be emphasized that markers should not be expected to disappear immediately after tumor removal. The level at a particular time after therapy would reflect the original level and the length of time that the markers would remain in circulation. Thus, when elevated markers are detected after surgery, their significance can be evaluated only by obtaining additional specimens over a period of time. Furthermore, if the test is for reactive markers rather than for circulating levels of tumor-produced antigen, the disappearance after curative resection might not be expected. In fact, with some assays of reactive markers, the levels might increase after tumor removal.