Genetic and Phenotypic Markers of Tumors

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PREFACE

The study of tumor markers is not only one of the most important but also one that offers one of the richest perspectives in biology and clinical oncology. The aim of scientists in this field is to adduce evidence of a property that is typical of and exclusive to tumor cells, and which is easy to determine, in order to immediately recognize, or even better, to foresee, neoplastic transformations.

Unfortunately, despite the large number of scientists and laboratories engaged in this work, the ideal tumor marker has not yet been identified. However, it is worth noting that new trends in molecular biology and immunovirology have recently opened up new avenues that may lead to the eventual resolution of this problem.

In this book, different approaches to the identification of tumor markers, from the points of view of biochemistry, immunology, and molecular biology, are compared in order to explore possible interrelationships and to stimulate scientific collaboration among scientists active in these fields, both in basic research and in clinical applications.

We wish to thank all the contributors and also the publisher, especially Dr. Robert Andrews, for making the publication of this book possible.

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PHENOTYPIC AND GENETIC MARKERS OF CANCER: TURNING POINT IN RESEARCH

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1. INTRODUCTION

Cancer, after cardiovascular diseases, is the second cause of death both in Europe and U.S.A. Despite efforts and improvements in many fields of tumor research, the surviving time of cancer patients is strongly related to the stage of disease more than to therapeutic strategies.

For instance, if the five-years survival for colorectal cancer is 42% (average of all stages), the survival is about 90% for stage I patients (Miller, 1976). Therefore, screening procedures for early diagnosis of asymptomatic patients have been proposed, ranging from biochemistry (oncodevelopmental proteins) to molecular biology (onc-genes), from imaging (thermography) to the detection of so called "groups at high risk for the disease" (identification of associated diseases, family history, HLA). From these studies the term " marker" has been introduced for indicating a sensitive, specific, easy to perform, recognizable substance, related to the presence of the tumor.

The Bence Jones protein has been known for long time as the typical marker of myeloma; from this basis other proteins (enzymes, hormones, etc.) have been described and quantitative analysis has shown variations of their level in body fluids

(blood, urine, milk, cerebrospinal fluid etc.).

The carcinoembryonic antigen (CEA), alfa fetoprotein, polyamines, enzymes, protein or isomeric proteins, cell surface markers, have been extensively studied and charged of significance. They are expression of neoplastic progression rather than of cell transformation. In fact, changes occur in phenotypic products of transformed cells and the relationship between these markers and cancer is generally restricted to a statistical and/or clinical significance without a close analysis of the molecular biology of production or disappearance. Again they are often common to many tumor types or to inflammatory diseases, and the variation of their level occurs only in clinically diagnosticable patients.

In this paper we briefly summarize data and problems about the most popular biological markers of tumors and we point attention to those markers which seem to be good candidates for an involvement in the cell transforming events.

2. SENSITIVITY AND SPECIFICITY

A marker should have the following characteristics:

a - sensitivity, to give positive results in patients bearing a tumor;
b - specificity for the tumor in question (hystological type, grade of malignancy);
c - availability in body fluid or tissue specimens (i.e. fine needle biopsies);
d - early appearance or loss, so that the early detection is significant for a very small tumor burden;
e - measurable levels which vary with the increase of tumor mass or recurrence of effective curve;
f - good feasibility (easy to perform, low cost, etc.).

Unfortunately the above mentioned requirements are difficult to assess for a single biological marker. The validity of each marker is dependent from its sensitivity and specificity. Sensitivity is the ability to give a positive result in patients bearing the disease (true positives), whereas specificity is the

ability to give a negative result in healthy (regarding the disease) patients (true negatives).

Regarding a test, we may have the following possibilities:

-true positive (A)
-true negative (B)
-false positive (C)
-false negative (D)

Sensitivity is expressed by A/A+D (true positive/total patients). Specificity is expressed by B/B+C (true negative/total healthy patients). Predictiveness is expressed by A/A+C (true positive/true+false positive)

A test with good sensitivity has often a low specificity and this fact is more evident for tumor markers, such as oncodevelopmental antigens, which are present in patients bearing different tumors (i.e. carcinoembryonic antigen in colorectal, gastric, breast cancer, etc.). In this case predictiveness is low because of the incidence of false positives regarding that tumor (Cole and Morrison, 1980).

Simultaneous use of several tests as well as more advanced and selective methods (monoclonal antibodies as more specific binding molecules) can improve both sensitivity and specificity. Particularly, in the simultaneous use of several combined tests, significancy can be represented (Tab. 1) and the potential use of multiple markers for the management of cancer patients can be studied. Screenings of asymptomatic patients at risk by using multiple tests have been proposed as potentially more effective means for detecting early colorectal cancer (Fath Jr. and Winawer, 1983).

However, there are still very few diagnostic tests that are useful to the oncologist. Despite millions and millions of assays, results often are not reliable or useful because of technical problems, such as low selectivity, discrepancy between cost of large scale programs and early diagnosis, uncorrect clinical use (Herberman, 1983). Thus, development of new markers, preparation of monoclonal antibodies, elucidation of proteins and onc-genes sequences lead to a new step in the study of more sensitive and specific markers, such as those which are known to be related to the transforming process (Tab. 2).

Table 1. Sensitivity, Specificity and Predictiveness for a single test and for tests' series

A=true positive	B=true negative
C=false positive	D=false negative
SINGLE TEST	
Sensitivity	$A / A + D$
Specificity	$B / B + C$
Predictiveness	$A / A + C$
SEVERAL TESTs	
Sensitivity	$\frac{A_1+A_2+A_n}{n} / \frac{A_1+A_2+A_n}{n} + D$
Specificity	$B / B + \frac{C_1+C_2+C_n}{n}$
Predictiveness	$\frac{A_1+A_2+A_n}{n} / \frac{A_1+A_2+A_n}{n} + \frac{C_1+C_2+C_n}{n}$

Table 2. Expectation of improvement by using genomic markers of tumors

Marker type	A	B	C
Phenotypic markers i.e. enzymes, proteins, oncodevelopmental proteins, etc.	+	+	+++
Markers related to cell transformation i.e. detection of Ig related to EBV, Oncorna viruses, etc.	+	++	+++
Genomic markers i.e. detection of EBV, Oncorna viruses, onc-genes, etc.	+++	+++	±

A = Specificity B = Sensitivity C = Easy to perform

3. TUMOR ASSOCIATED MARKERS

Tumor Associated Antigens (TAAs) and neoplastic cell metabolites (including proteins, enzymes, hormones, carbohydrates) which are present in tumor bearing patients are included in this category. The immunological detection of new shared antigens or the finding of increased levels of proteins (such as oncodevelopmental antigens, enzymes, etc.) should be considered together since these compounds are present also in non neoplastic patients and their absence in normal tissue at any time of development is not demonstrated yet . In fact, by using sensitive techniques (RIA, ELISA), many antigens have been found in traces in normal, not embryonic cells.

Thus, TAAs and other phenotypic markers can be used by the oncologist when one of the following conditions occur:

a - quantitative differences in expression in tumor cells versus normal cells (i.e. enzymes);
b - presence in normal tissues, but only in a particular type of tumor (i.e. ectopic hormones);
c - presence in embryonic normal cells, but only in traces in adult healthy cells (i.e. AFP);
d - qualitative differences from normal proteins (i.e. isoferritin).

Low level/high level or high level/low level or typic pattern/atypic pattern between normal and tumor patients are the required findings regarding these markers. In this section we briefly discuss patterns and clinical applications of this type of tumor markers.

3.1.Oncodevelopmental proteins

The presence of tumor antigenicity in syngeneic systems has been a means for identification of tumor "diversity" and for

improving tumor diagnosis and monitoring. The presence of an alpha-fetoprotein (AFP) in the sera of patients bearing primary hepatocellular tumors was the first record of a significant variation of the presence of a fetal protein in adults (Abelev et al., 1971). Thus, antigens purified from colon cancer were characterized after rabbit immunization and absorption with normal colon or blood components. These antigens were not found in adult tissues except than in malignant tumors of gastrointestinal tract and pancreas. The same antigens were found in fetal gut, liver and pancreas between 2 and 6 months of uterine life. Thus, the term " carcinoembrionic antigen" (CEA) was introduced and the progress in the measurement of traces by using RIA and ELISA methods extended the CEA-test in monitoring cancer patients (Fath and Winawer, 1983; Holyoke et al., 1982). These antigens are present in malignancies different from the original. CEAs have been found either in serum or cytosol of mammary and lung cancer, of gynecologic and urological malignancies and soft-tissue tumors (Duffy et al. 1983; Lee, 1983; Holyoke et al., 1982). The research on this field focused attention on a variety of markers and proteins such as fetal sulfoglycoprotein (FSA), colonic mucoprotein antigen (CMA), colon specific antigens (CSAs),colon specific antigen p (CSAp), pancreatic oncofetal antigen (POA), Tennessee Antigen etc, have been described. They are only in few cases cell specific, not organ or cancer specific (Goldenberg, 1982). On this field, excellent articles have been included in specific books (see General Bibliography).

The problems raising from the above presentation, according to the aim of this paper, are the following:

a - when more than a single test is performed, a test can be true positive when the other one is true negative and viceversa (e.g. CEA and CSAp correlation is about 50-60%);
b - presently RIA and ELISA tests use policlonal antibodies and they can be improved by the use of monoclonal antibodies;
c - the use of radioactive antibody combinations for detecting tumor localizations (e.g. Ig mixtures against CEA and CSAp) can be of great advantage;
d - the use of more than a single test can improve predictiveness of such markers.

3.2. Proteins

Differences in protein levels or enzyme activities between healthy and cancer patients have been described. Immunoglobulins, Ferritin and Isoferritin, Ceruloplasmin, alpha 1-Antitrypsin, beta 2-Macroglobulin, Haptoglobulin, C-reactive protein, Circulating Immune Complexes (CIC), have been measured in serum of cancer patients and they can be of help in staging or follow up. Biochemical abnormalities are different in the various tumors and the serum level of these markers correlate with tumor size. Thus, they cannot be used for early diagnosis. Only serum ferritin is a candidate for early diagnosis purpose. The level in normal subjects ranges between 10 and 200 ng/ml and higher values have been observed in about 2/3 of patients with lung cancer, but also in 15% of healthy smokers.

However, when data of lung cancer patients have been disaggregated for stage of disease, values observed in stage I (645 ng/ml; 81% elevated with cut-off level to 200 ng/ml) have been higher than in stage II (472 ng/ml; 53%) and in stage III (487 ng/ml; 57%). The fact that higher levels occur in stage I and that values in normal subjects never exceed the limit of 200 ng/ml, suggests that this test is a good candidate for early detection of lung cancer (Urushizaki and Niitsu, 1982). Except than the cases of ferritin and of Ig in myeloma, protein measurement cannot be used for early diagnosis, but only for monitoring tumor progression. The fact that about 15% of healthy smokers had high level of ferritin is suggestive for high risk patients and/or early detection of occult cancer.

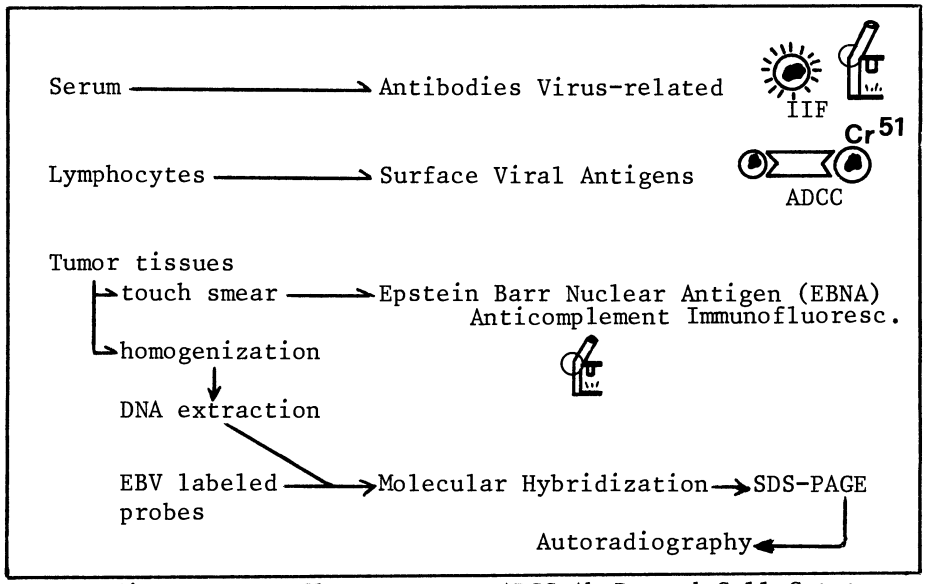
3.3 Enzymes

The use of enzyme assays is very popular in clinical chemistry because easy to perform. In cancer monitoring, extent, stage, progression or regression of the disease have been related to the level of enzymes.

Tab. 3 summarizes the most important enzymes described in literature. Although research on enzymes as tumor markers is older than for other ones, they are of little help in early diagnosis

Table 3. Variation of the level of enzymes in Tumors bearing patients

Enzyme	Tumor	Variation
Amylase	Lung	+
Aryl Sulfatase B	Colon	+ urinary
Glycosyltransferases		
Fucosyltransferase	Breast, Colon	+
Galactosyltransferase	Colon, Ovary	+
Sialyltransferase	Massive metastatic tumors (in Lung or Liver)	+
α-Glutamyl Transpeptidase	Hepatoma, Liver Metastases	+
Hexosaminidase B	Colon	+
Histaminase	Medullary Thyroid C. Lung (Small Cell C.)	+
Lactic Dehydrogenase	Liver Metastases	+
LDH_5	Breast, Brain, Lung, Uterus Prostate, Kidney, Stomach	+
Lysozyme	Acute Myelomonocytic Leuk	+
	Acute Myeloblastic Leuk	+
Placental Alk.Phosphatase	Germ cell	+
Prostatic Acid Phosphatase	Prostate	+
Ribonuclease	Pancreas, Ovary, Epidermoid cancer	+



IIF=Indirect Immunofluorescence; ADCC=Ab Depend.Cell.Cytotox.

Fig. 1 - Detection of Epstein Barr Virus (EBV) infections by specific Immunoglobulins and DNA internal repeats

and their relevance can be restricted to a few cases (i.e. phosphatases).

3.4. Steroid receptors

Biological basis of endrocrine therapy is the presence in the cells of specific receptors, which represent the way by which tumors are responsive or not to the administration of hormones or inhibitors. Binding capacity (for estrogen > 10 fentomoles of steroid bound/mg cytosol protein) and dissociation constant (usually for estrogen-receptors complexes ranging between 10^{-10} and 10^{-11} M) should be analyzed together.

About 60% of primary breast cancer and 50% of metastatic one are positive for estrogen receptors and about 50% of primary breast cancer is positive for progestin receptors. These positivities are related to the clinical response to endocrine therapy and positivity is also a prognostic factor. Many molecular species of receptors have been isolated and the 6S and 8S components are related to the responsiveness. Unresponsive cells show the 4S component, even under conditions of low ionic strength. Steroid receptors are present in various tumors other than those which exhibit sexual dimorphism (i.e. breast, uterus). Colorectal cancer for example is positive for estradiol. However, steroid receptors are useful for therapeutic strategies only.

3.5 Ectopic Hormones

The production of ectopic hormones by tumors occurs frequently and RIA for hormones or precursors of hormones or hormone-like substances have been introduced. A typical example is small cell carcinoma, which often produces ACTH/LPH related molecules. Usually tumors derived from an endocrine tissue produce normal hormones, whereas ectopic hormones are heterogeneous, with a presence of high molecular weight molecules, fragments, subunits, abnormal breakdown products. Lung tumors producing ACTH-like molecules have been described. They can produce "big ACTH" (20,000 M.W.), ACTH 1-39 or ACTH 2-38 or ACTH 18-39 small M.W. components, beta (1-91) LPH and Y(1-58) LPH,

alpha 61-76 and beta 61-91 endorphins or calcitonin, as expression of both genomic derepression and "maturation" defects. As tumors markers, abnormal or ectopic hormones seem to be more interesting than hormones produced by endocrine tissues (Odell and Wolfsen, 1978).

However, criteria to establish ectopic hormone production have been outlined to prevent untrue evaluations:

1- arteriovenous hormone gradient across tumor mass;
2- in vitro production by the tumor;
3- demonstration of the hormone in the tumor at levels higher than in adjacent normal tissues;
4- quantitative and/or qualitative differences between the product of the tumor and the same hormone from normal endocrine tissue;
5- fall/rise of the level related to the regression/progression of the tumor.

By these criteria, tumors derived from lung (FSH, LH, Calcitonin, Growth Hormone, Vasopressin, HCG, ACTH, beta-Lipotropin), uterus (vasopressin), kidney (Parathyroid Hormone), liver (PTH, Somatomedin), thymus (ACTH, beta-MSH, PTH, Calcitonin) have been described as ectopic hormone production.

It was suggested that findings of ectopic hormones may facilitate the detection of both primary and metastatic neoplasia. As discussed by Neville (1982), various limitations, like those found for oncofetal antigens, have restricted the role of these markers. In fact only the assay of qualitative abnormal products can be used for early diagnosis, but unfortunately RIA tests generally do not discriminate between normal and abnormal polypeptide hormones. However, the monitoring of ectopic hormones levels after primary diagnosis of tumor can be used in the follow-up of the patient. If RIA test specific for abnormal products shall be introduced, the significance of ectopic hormones should be reconsidered.

3.6 Miscellaneous

Other markers have been proposed. Urinary and plasma levels

of polyamines (putrescine, spermidine and spermine) have been related to tumor reduction. Increase of urinary level of putrescine 24hrs after chemotherapy, correlates with a clinical response. These markers can be used for monitoring but not for diagnosis (Russell and Durie, 1978).

Serum protein-bound carbohydrates (fucose, mannose, and galactose) or lipid-bound sialic acid have been described to be correlated respectively with the presence of lung small cell carcinoma or with various tumors as cancer of the prostate, bladder, breast, lung, colon, ovary, and in leukemia, lymphoma, Hodgkin disease and melanoma. These markers lack the required sensitivity and specificity for routine cancer detection and they can be used only for help in clinical evaluation of tumor progression. Other markers (i.e. breakdown products of tRNA: Cimino F. et al., this book) have been proposed. They are generally useful for monitoring cancer patients rather than for early diagnosis, because unrelated to the transforming events.

4. PHENOTYPIC MARKERS AS EXPRESSION OF CARCINOGENESIS

As discussed in the first part of this review article, biomarkers which are expression of neoplastic transformation seem to be inadequate for precocious biochemical diagnosis of tumors. For this purpose a marker should be more sensitive and more specific: such a marker should be expression of oncogenesis rather than a product of an established neoplasia.

New approaches in this direction seem to be:

4.1- Immunological detection of new-shared antigens

Transforming events by viruses have been joined to the advent of new specific antigens. A diagnostic procedure for detecting new shared antigens induced by Epstein-Barr virus infection is shown in Fig. 1. Membrane antigens (MA) and nuclear antigens (EBNA) of the Epstein-Barr virus can be detected in patients with Burkitt lymphoma and nasopharyngeal carcinoma. The most prevalent EBV-associated antibodies are IgA against virus capsid antigens,