Overview of Oncofetal Antigens in Cancer

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ABSTRACT

Oncofetal antigens are substances which are produced by tumors and also by fetal tissues but they are produced in much lower concentration by adult tissues. The oncofetal antigens which have been identified are reviewed. The relevance of alpha - 1 - fetoprotein (AFP) and carcinoembryonic antigen (CEA) in neoplastic disease are summarized. Elevated serum concentrations of AFP have been principally associated with primary liver cell cancer (82 percent) and with ovarian and testicular tumors which contain yolk sac tumor cell elements. Quantitation of the serum concentration of CEA can be used as an adjunct for the diagnosis and staging of colon cancer patients and for the post operative follow up of patients for tumor recurrence. The possible role that mouse monoclonal antibodies will play in the characterization of oncofetal antigens is reviewed. Some of the difficulties which may arise when mouse monoclonal antibodies are used to define clinically relevant oncofetal antigens are reviewed.

Introduction

Cancer biologists have for many years appreciated the similarity between fetal tissues and adult malignant tissues. Most recently over the past 17 years with the development of the field of oncofetal antigens, it has become apparent that numerous cellular constituents normally present during embryonic life may also be present during fetal and neonatal life. These same cellular constituents may be present at much lower concentrations in adult tissues. These facts and the marked resemblance observed between immature and cancerous cells at other levels of biological activity and structure have led many to conclude that spontaneous and experimentally induced malignant tumors may arise from incomplete differentiation or by retrodifferentiation.

A brief review of oncofetal antigens cannot possibly do justice to this ever expanding area of tumor biology. The reader should refer to the two recent publications which discuss tumor markers and to recent comprehensive reviews. The majority of the information which will be presented in this manuscript is contained in these references and, therefore, no specific attempt will be made to reference the data which are summarized in these other publications.

When it became apparent that some
substances produced by tumors could also be identified in fetal tissue, the obvious hope was to develop assays which would allow the detection of the presence of cancer before it was clinically detectable. There are several criteria which any biological tumor marker assay should meet to be considered clinically relevant. These include: (1) the assay should be simple, reproducible, widely available, and cost effective, so that it can be easily applied to a large number of patients; (2) the assay should detect a quantitative difference between those with and without cancer; (3) the assay should be highly sensitive; that is, it should detect a large number of those affected and simply stated, it should have few false negatives; (4) it should be useful for monitoring the long term course of the tumor and thus provide early detection of recurrence; and (5) it should be specific, produce few false positive values, and indicate the site of organ of cancer involvement.

There have been several attempts to define oncofetal antigens which can meet these criteria. The most well known being alpha-1-fetoprotein (AFP), tissue polypeptide antigen (TPA), fetosulfoglycoprotein (FSA), pancreatic oncofetal antigen (POA), and carcinoembryonic antigen (CEA). Since AFP and CEA are two of the best known oncofetal antigens, they will be discussed in some detail.

**Alpha-1-fetoprotein**

The principle facts about the AFP can be summarized in table I. There has been confusion about the testing for elevated levels of AFP in patients' serum in those suspected of having a malignancy. After personally assaying nearly 3,000 serum samples, it was possible to develop practical and straightforward criteria for the use of the AFP assay in the diagnosis of hepatocellular carcinoma and of germ cell tumors. It is the conviction of the author that if a reproducible radioimmunoassay for AFP (or an assay with a similar sensitivity) were made available routinely to physicians in this country, it would make a positive contribution to the diagnosis and treatment of malignant disease.

Somewhat hidden in the principal facts about AFP are several important findings. With regard to hepatomas, it is important to remember that approximately 15 percent of hepatomas on a worldwide basis are AFP negative. Therefore, a normal AFP level of <20 ng per ml does not completely exclude the diagnosis of hepatoma, although it makes it less likely. The early diagnosis of small hepatocellular carcinoma may make it possible to do curative surgical resection of the tumor. This approach is feasible in some areas of the world, such as China, but it is unlikely to be successful in the USA because such a high percentage of hepatomas develop in cirrhotic livers. These patients, by the very nature of their illness, constitute a "high risk" group making surgical interventions unlikely. With germ cell tumors, it is again important to remember that the return of AFP to normal levels may not correlate
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TABLE II

Little Known Facts About Alpha-1-fetoprotein

1. Alpha-1-fetoprotein (AFP) has been shown to cause immunosuppression occasionally. The exact mechanism is yet to be elucidated.\(^5\)
2. A small amount of AFP is bound to the membrane of AFP secreting tumor cells.\(^1\)
3. Antibody to AFP can be used to kill AFP positive tumor cells both in vitro and in vivo.\(^12,13\)
4. Antibody to AFP when conjugated to Daunomycin, ricin, or other agents can be used effectively to kill tumor cells in vitro and in vivo.\(^14\)
5. Antibody to AFP which has been radiolabelled can localize to AFP positive tumors.\(^15\)

with the disappearance of tumor as AFP serum levels will only be elevated if the tumor contains yolk sac derivatives.

There are several lesser known facts about AFP which are well documented in the literature, but which have been given considerably less attention. These are listed in table II. These “little known” facts on AFP point out that to a certain extent the “oncofetal” antigens and their antibodies are being asked not only to serve as a diagnostic test for early tumor growth and for the early detection of tumor recurrence but also as therapeutic reagents. With more recent data available, it is hopeful that antibodies to these “oncofetal antigens” can be utilized as reagents to localize tumors which are producing these proteins. It is also hoped that antibody to these “oncofetal proteins” may also be utilized to deliver toxic and/or chemotherapeutic agents to these tumors. The preliminary results in animal models, with antibody to AFP, have clearly shown that both of these approaches are highly feasible and very early clinical data further support the concept that antiserum to AFP and other “oncofetal antigens” may be useful in tumor site localization and in the therapy of malignancy.

Shortly after the description of AFP, Gold and Freedman\(^5\) described another protein which was associated with fetal tissue and also produced by neoplastic tissue. This antigen was found to be present in the plasma of patients with carcinoma of the colon and initial studies showed that it seemed to be specific for the diagnosis of adenocarcinomas of the colon. This antigen is the now famous CEA.

Carcinoembryonic Antigen

A considerable amount of work has been done to clarify the relationship of CEA to neoplastic disease and the reader is again encouraged to refer to more complete reviews\(^1,3,6,12\) for specific details concerning this work. Several statements can be made about CEA and neoplasia:

1. High serum levels of CEA have been demonstrated to be present in association with colon carcinomas, but CEA determinations cannot serve as a screening test for the early diagnosis of colon cancer.
2. Higher serum levels of CEA are seen with more advanced tumors: Dukes D—85 percent positive, Dukes C—75 percent positive, and Dukes A—30 to 38 positive.
3. With Dukes B and C lesions, the preoperative levels of CEA titers which are above 5 ng per ml have been associated with an earlier recurrence rate. For example, when the CEA serum level was <5 ng per ml, the mean interval to tumor recurrence was 30 months, whereas when the CEA serum level was >10 ng per ml the mean interval to recurrence was 9.8 months.\(^3\)
4. The most promising clinical application of CEA determination has been to follow carcinoma patients post-operatively for tumor recurrence. The lead time before recurrent tumor was detected clinically has been from three months to three years. There is a 7 percent false
negative rate which implies that recurrent tumor can occur with no significant elevation of the serum CEA.\(^4\)

5. Serum levels of CEA can be utilized to follow responses to chemotherapy as serial CEA titers tend to correlate with the tumor responses.

6. Increased CEA levels have been associated with other tumors with the highest association with pancreatic carcinomas (85 to 90 percent).\(^3\) Other tumors which have been found to produce CEA include lung, breast, prostate, urinary bladder, kidney, and ovary.

It should be emphasized that elevated levels of CEA are not necessarily diagnostic of colon cancer. The largest majority (96 to 97 percent) of healthy non smokers will have CEA titers which are less than 2.5 ng per ml. A portion of active smokers (20 to 40 percent) will have CEA values which are greater than 2.5 ng per ml, and a significant percentage of these patients have serum concentrations which are greater than 5 ng per ml. Elevated CEA levels have been seen with intestinal obstruction, biliary obstruction, uremia, pancreatitis, cirrhosis, colorectal polyposis, ulcerative colitis, regional enteritis, and peptic ulcer disease. In this group of patients ~25 percent of the patients will show transient elevations of their serum CEA levels.

Carcinoembryonic antigen has been demonstrated to accumulate and localize to the cell membrane, but it has also been demonstrated in the cytoplasm of the cell. Recently, attempts have been made to locate the site of CEA positive tumors using antibody to CEA which has been radiolabeled with iodine. The largest amount of work has been done by Goldenberg\(^6\) from the University of Kentucky. In his studies, Goldenberg uses subtraction methods whereby the computer subtracts the energy of technetium (140 Kev) from that of iodine (364 Kev) in order to compensate for the blood and other non target areas of radioactivity. Goldenberg has examined patients who have many different types of tumors which have been shown to produce CEA. With colon carcinoma patients, he has been able to detect 10/12 primary colon tumors and 49/53 metastatic tumors. These results are very encouraging; however, at the present it is unclear to what extent this technique can improve upon other more conventional methods of testing, such as radionuclated scanning, CAT scanning, etc. There is the very clear hope for the future that antibody-toxin or antibody-chemotherapeutic conjugates can be prepared which will localize to CEA positive tumors and result in selective tumor cell destruction and cell death with a prolongation of the survival of the host.

**Monoclonal Antibody Techniques**

The interest in tumor associated antigens and in oncofetal antigens has increased with the development of the monoclonal antibody techniques. This technique has made it possible to develop a very large number of antibodies which have the ability to identify unique antigenic determinants (epitopes) which are present on cells or which are produced by cells. These techniques have great potential for the development of diagnostic tests for malignancy and for the development of selective seroimmunotherapeutic reagents for cancer treatment. Because of this potential, a major scientific industry complex, which is based on monoclonal antibody techniques, has been developing over the past several years. A major amount of money, personnel, and dedicated scientific effort is now being put forth to identify and to characterize monoclonal antibodies which
may be useful in cancer diagnosis and treatment.

The questions which have been asked by previous studies, which used xenogeneic antiserum in an attempt to characterize tumor associated antigens, still remain the same questions for those people using monoclonal antibodies. Three such questions are as follows: (1) to what extent are antigenic determinants which are detected by monoclonal antibodies present on cancer cells, fetal cells or on adult cells? (2) to what extent can monoclonal antibodies be utilized to develop diagnostic tests for the “early” diagnosis of cancer or tests which can be utilized in the therapeutic monitoring of patients who have cancer? and (3) can monoclonal antibodies, which appear to define an antigenic specificity which is present on cancer cells and not on normal cells, be utilized as seroimmunotherapeutic reagents in the treatment of cancer?

Although it is a bit premature to attempt to answer these questions, early investigative studies have provided some interesting information about monoclonal antibodies and their application to cancer studies. Monoclonal antibodies have been developed to some of the classical “oncofetal antigens”. In the case of a relatively homogenous antigen such as AFP, it appears that the monoclonal antibodies have limited advantages over xenogeneic antiserum in the diagnostic radioimmunoassays or in the seroimmunotherapy of cancer. In the case of the heterogenous antigens such as CEA, it is the hope of a number of researchers that the monoclonal antibodies may be useful to characterize further the antigen and to be able to improve the diagnostic specificity of the various radioimmunoassays. The majority of monoclonal antibodies which have been developed so far to leukemia associated antigens, to malignant melanoma antigens, and to other cancer cells appear to define differentiation antigens which are present on cancer cells, some embryonic cells. To a limited extent, these antigens may also be present on normal cells. The most successful immediate application of the monoclonal antibody technique has been to define antigens which are present on adult lymphoid populations of normal cells.

In an attempt to circumvent some of the problems which were mentioned previously, there has been considerable interest in the development of a human-mouse hybridoma system or a human-human hybridoma system. Both of these systems are currently in their scientific infancy, and it is too premature to speculate if they will be the best methods for the characterization of tumor associated antigenic determinants. A distinctly different approach has recently been taken by Irie and Katano of UCLA. They have recently described the production of an antibody to the oncofetal antigen OFA-I by peripheral blood lymphocytes (PBL) which were transformed by Ebbstein Barr Virus (EBV) to human B-lymphoblastoid cell lines. Out of 232 EBV transformed PBL specimens, two long term lines, L55 and L72, were established; they produced IgM anti - OFA - I for six months and more than 12 months. Antibodies for L55 reacted with various human cancer cells, including melanoma, sarcoma and carcinomas, whereas antibody from L72 reacted with tumors of neuroectodermal origin (melanoma, glioma, and neuroblastoma). Since antibody to OFA-I can be cytotoxic to OFA - I - positive cells, Irie and Katano suggest that the antibody OFA-I produced by L72 may be a potential reagent for specific immunotherapy in patients with tumors of neuroectodermal origin.

Summary

In summary, the concept of oncofetal antigens appears to have an increased relevance in cancer biology. These pro-
Weptics can clearly serve as diagnostic markers of tumor development and growth. Serum levels of the serum concentrations of these proteins can be utilized as a marker protein for following a patient for tumor recurrence. In addition, it has been demonstrated that specific antibody conjugates may be utilized to determine the site where a tumor is present. Preliminary studies in animal models strongly suggest that specific antibody conjugates may be utilized to deliver toxic and/or chemotherapeutic compounds to an "oncofetal" protein producing tumor. This therapy will kill the tumor cells and result in host survival. It is my personal belief that the further characterization of oncofetal proteins may help to further elucidate the nature of tumor development as a phenomenon of retrodifferentiation or arrested differentiation. Furthermore, studies on these proteins should also help to provide information about the fetal maternal relationship.

References