Monoclonal Antibodies to Detect Markers Specific for Tumors

J. V. KLAVINS, M.D., PH.D.

Department of Pathology,
The Catholic Medical Center of Brooklyn and Queens, Inc.,
Jamaica, NY 11432

ABSTRACT

Monoclonal antibodies to different markers can facilitate the diagnosis of T and B cell lymphomas, histiocytic lymphomas, malignant histiocytosis, and Hodgkin's disease. The B-cell lymphomas can be identified specifically by monoclonal anti-idiotype antibodies. Monoclonal antibodies are produced to defined markers like carcinoembryonic antigen (CEA) in colon carcinomas and other antigens especially of breast and ovarian carcinomas. When conjugated with 123I, monoclonal antibodies can be used to detect tumors by emission computerized tomography. Chromogranins are markers for neuroendocrine tumors, and they can be identified by monoclonal antibodies. More recently monoclonal antibodies have been produced to ras gene product p21, present in breast and colon carcinoma cells.

Introduction

Since monoclonal antibodies are highly specific, it is possible to study various gene products in neoplasia that would be unique or considered specific markers for cancer. One way to do this is to develop monoclonal antibodies to already known markers; the other approach is to produce monoclonal antibodies not knowing the gene products at all. Consequently, the strategy in monoclonal antibody production is to immunize mice with cancer cells to produce monoclonal antibodies.

Monoclonal antibodies have been used as reagents in the analysis of well defined tumor markers, such as alpha fetal protein, carcinoembryonic antigen, human chorionic gonadotropin and others. They are more consistent in their reproducibility and in reactivity with antigens than polyclonal antibodies.

Monoclonal antibodies have been produced against antigens in a variety of tumors in expectation of detecting an antigen as a marker. Such antibodies may represent any of the three groups of tumor markers, — oncofetal, tumor-associated, or tumor-specific. Furthermore, they may be universal markers or markers with a limited distribution.

The undefined antigens discovered by monoclonal antibodies in initial studies were suggested as specific markers for a respective neoplasm. After more extensive investigations, they appeared as oncofetal or tumor-associated antigens.
The only tumor-specific marker of clinical significance at the present time is the idiotype of each B-cell lymphoma clone.\( ^{18} \)

**Markers for Lymphomas**

There are numerous cell markers for the T and B cell lymphomas for their recognition and classification. Recently, monoclonal antibodies have also been produced against those lymphomas which are derived from histiocytic-monocytic cell lines.\( ^{10} \) These monoclonal antibodies can facilitate the diagnosis of histiocytic lymphoma, malignant histiocytosis, and Hodgkin's disease. These monoclonal antibodies did not react with normal B or T lymphocytes or lymphomas derived from these types of cells. However, they reacted with nuclear membranes of their corresponding normal histiocytes and interdigitating reticulum cells in normal lymph nodes.

**Monoclonal Antibodies to Idiotype of B-Cell Lymphoma**

The idiotype of each B-cell lymphoma clone differs from all normal cells of the patient. Thus, it represents a specific tumor marker\( ^{11} \) with a limited distribution.\( ^{12} \) The immunoglobulin on the cell surface appears as a monoclonal entity with a single light-chain type and the variable region specific for each patient.

**Production of Anti-Idiotype Antibodies**

The procedure for the production of monoclonal anti-idiotype antibodies is fusion of malignant human B cells with myeloma cells. These B cells are not secreting immunoglobulin. After the fusion, such hybridomas produce B-cell surface immunoglobulin. This immunoglobulin is then used to immunize mice for the production of anti-idiotype antibodies by the hybridoma technique. Another method with a shorter time for the production of monoclonal anti-idiotype antibodies is one step immunization of the mice with intact lymphoma cells.\( ^{19} \) The frequency of anti-idiotype-specific hybridomas with this approach was approximately one percent. A four-layer enzyme linked immunosorbent assay (ELISA) screening was effective to detect this low frequency of hybridomas specific for idiotypes. There was no need for previous purification of the tumor cell immunoglobulin. The tumor cell lysate was the source of the idiotype target.

Such anti-idiotype antibodies can be used to identify malignant cells specifically and differentiate them from the other types. Thus, circulating tumor cells could be detected, while it was not possible with the routine methods. They also appeared effective in the treatment of a patient with B-cell lymphoma.\( ^{18} \)

It has been observed that variants of idiotypes may develop as a result of somatic mutation in the variable region of the corresponding genes.\( ^{17} \) Therefore, more than one species of monoclonal anti-idiotype antibodies is needed in such situations to identify all malignant cells.

An interesting feature is the formation of an antigenic image within the anti-idiotype antibodies.\( ^{8} \) This was demonstrated with the following sequence of immune reactions. First, a mouse monoclonal antibody (Ab1) was produced to an antigen of a human gastric carcinoma. To this antibody, an anti-idiotype antibody was raised in goat (Ab2). The Ab2 was then used to immunize both mice and rabbits, which produced an anti-anti-idiotype antibody (Ab3). This Ab3 has the identical binding properties as Ab1. Therefore, it can be concluded that Ab2, the anti-idiotype antibody, has an internal structure resembling or identical with the gastric carcinoma antigen.
Human Monoclonal Antibodies

There are many attempts to develop human monoclonal antibodies. One approach is to produce them by the Epstein-Barr Virus (EBV)-hybridoma technique. The EBV induces indefinite division of donor B cells and, therefore, they can be used for repeated fusions. A fusion partner also has been developed. The KR-4 cell line has been selected as the fusion partner. It meets the necessary criteria for an effective partner by improving 10 to 100 fold the hybridization frequencies of EBV transformed donor lymphocytes.

Another approach to produce monoclonal antibodies of human origin is using a human lymphoblastoid B cell line. It is then fused with human B cells to produce hybridomas secreting human monoclonal antibodies. These human-human hybridomas can contain human B cells which are reactive with tumor associated or specific antigens. For diagnostic methods, for example, tumor imaging is necessary to produce large amounts of such antibodies. They are produced by some commercial companies, using mass culture technology.

Monoclonal Antibodies for Emission Computerized Tomography

Monoclonal antibodies to defined antigens can be used for the detection of tumors by emission computerized tomography. So far, the best results have been obtained by using Fab fragment of a monoclonal antibody Mab 35. This monoclonal antibody was raised against carcinoembryonic antigen and conjugated with 123I. Tumors less than two to three cm in diameter could be detected. The application of this method still needs to be compared with other techniques, such as computer associated tomography scanning and nuclear magnetic resonance. At the present time, in comparison with different monoclonal antibodies and tracers, Mab 35 labelled with 123I appears to be the best approach to determine the size of tumors in colon carcinoma patients.

There are other malignant neoplasms which have been investigated by this approach, such as germ cell neoplasms, thyroid carcinomas, insulinomas, renal cell carcinomas, melanomas, and breast carcinomas.

Monoclonal Antibodies to Carcinoma of Breast

Monoclonal antibody 3B18 is a gamma 1 isotype. It was generated after immunizing mice with a human cancer cell line MCF7. This monoclonal antibody was specific in about 80 percent of all breast cancers. It did not cross react with normal, adult human tissues and other carcinomas. If further studies demonstrate that the antigen to which this monoclonal antibody is reacting is not the differentiation antigen expressed in the embryonic state, then indeed it might be considered as a tumor specific marker. However, the specificity of such an antibody can be questioned, since it also cross reacted with some cells in fibrocystic disease.

Another monoclonal antibody designated by Lemaistre as 323/A3 did not react with normal breast tissues but was present in 20 percent of fibrocystic disease tissues and in 61 percent of breast carcinomas. This antibody was also induced by MCF7 cells.

One such monoclonal antibody, HMFG-2 conjugated with 131I, has been used not only for diagnostic purposes, but also for treatment. It was used in patients with metastatic breast carcinoma and administered intrapleurally or abdominally, inducing in some cases remission of the disease.

Use of a pool of monoclonal antibodies is another approach in the detection of
cancer associated antigens. With a pool of nine monoclonal antibodies to breast carcinoma, reactivity was demonstrated in 100 percent of 21 breast carcinomas tested, and in 100 percent of seven serous effusions associated with breast carcinoma. However, such an approach obviously would decrease the specificity of the markers. For example, serous effusions not associated with breast carcinoma reacted at a rate of 84 percent with this pool of monoclonal antibodies.

Monoclonal Antibodies to Ovarian Carcinomas

Murine IgG1 monoclonal antibodies, designated by Bast et al as OC125, react with ovarian carcinoma cell antigen, CA125, which is expressed in about 80 percent of serous cystadenocarcinomas of the ovary. The expression of elevated levels of this antigen in patient’s serum can indicate tumor burden, as well as the recurrence of the disease. Elevated levels of CA 125 were observed 10 to 12 months prior to the primary diagnosis of ovarian carcinoma. For this test, commercial kits are available.

Chromogranins in Neuroendocrine Tumors

Chromogranins are soluble proteins of chromaffin granules. An acidic glycoprotein, chromogranin A, constitutes the largest component in this group. Monoclonal antibodies to chromogranin A together with polyclonal antisera against neuron-specific enolase are useful markers for pancreatic endocrine tumors. Chromogranin is present in glucagonoma, gastrinomas, in tumors producing multiple hormones, and in normal glucagon producing islet cells, but not in insulinomas. It was also demonstrated with a monoclonal antibody in other tumors with neuroendocrine cells such as neuroblastomas, paragangliomas, adrenal cortical carcinomas, medullary thyroid carcinomas, parathyroid adenomas, carcinoids of the stomach and small intestine, Merkel cell carcinoma of the skin, and normal neuroendocrine cells.

Monoclonal Antibodies to Oncogenes

Monoclonal antibodies to oncogenes, which can be considered as oncofetal tumor markers, have been applied to identify the malignant neoplasms. For example, monoclonal antibodies have been used to demonstrate ras gene product p21. This has been demonstrated in carcinoma cells of the breast and colon but not in dysplastic colonic epithelial cells or fibrocystic disease of the breast. There are some cells that had no expression of this gene product, indicating that maintenance of a transformed state is not necessarily related to p21 expression. A monoclonal antibody has also been developed for radioimmunoassay of the native p21, as well as the transformed one with the point mutation in NIH-3T3 cell lines. Again, it should be pointed out that the protooncogenes (e-oncogenes) are not specific tumor markers, since their products can be expressed in small quantities by normal cells.

References


