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Monoclonal Antibodies Reactive with Human Oncofetal Antigens

Masaharu KASAI[1,2,4], Tamotsu MIYAZAKI[1], Martyn W. BURK[3] and Hiroshi KOBAYASI[2]

[1] *The 3rd Dept. of Internal Medicine*
[2] *Laboratory of Pathology, Cancer Institute, Hokkaido Univ. School of Med., Sapporo, Japan*
[3] *Surgical Oncology, UCLA School of Med., Los Angeles, U. S. A.*
[4] *To whom correspondence should be addressed ; The 3rd Dept. of Interunal Medicine, Hokkaido Univ. School of Med., Kita 14, Nishi 7, Kitaku Sapporo, Japan*

SUMMARY

Monoclonal antibodies (MA) were developed following immunization of BALB/c mice with a human lung squamous cell carcinoma line UCLA-SO-P3(P3) and fusion of the spleen cells with mouse myeloma Sl94. Antibodies from hybridomas which showed preferential binding by ^{125}I-protein A binding assay to P3 cells rather than the autologous B lymphoblastoid line were selected and cloned. Three of these monoclonal antibodies demonstrate the presence of membrane antigens which react to a differing degree to human tumor cells and embryonic cells. MA806B4 is IgG2a, and reacts to carcinomas and embryonic cells but not to melanomas, sarcomas and lymphoblastoid lines. MA170C5 and MA754A3 are IgG2a and IgG2b, respectively, and react to carcinomas, embryonic cells and some of the melanomas, and also react at lower level to fibroblast. For protein analysis of antigens detected by these antibodies, immunoprecipitation using surface-iodinated and detergent solubilized P3 cells was performed and molecular weights (MW) of the antigens were analysed by SDS-PAGE. The MWs of the antigens for MA806B4, MA170C5 and MA754A3 were detected as 135 K, 130 K and 55 K, respectively. These antibodies did not share the same antigenic determinant and the antigens detected by them were different from carcinoembryonic antigen (CEA) by competition assay.

Key words: Monoclonal antibody, Lung carcinoma, Oncofetal antigen, Molecular weight, Carcinoembryonic antigen

INTRODUCTION

The antigenic relationships among tumors, fetal cells and normal cells are of

great interest not only for the study of oncofetal or oncodevelopmental markers but also for clinical diagnosis and therapeutic application. Intensive research to identify specific antigens on tumors or oncofetal tissues has been investigated(1–5), and hybridoma technology (6) has greatly contributed to the detection of antigenic determinants on tumors. In the past few years, we have developed monoclonal antibodies against human lung cancers and identified membrane antigens (7–9). Among monoclonal antibodies produced against human squamous cell carcinoma cells, three monoclonal antibodies react to carcinomas and embryonic cells and the antigens detected by them are discussed.

MATERIALS AND METHODS

Cell lines

Human lung carcinoma lines (UCLA-SO-P1, -P3, -P5, -P6, and -P7), melanoma lines (M14, M15, M16, M19, and M20), sarcoma lines (S1 and S3), B-lymphoblastoid line (PL3, L14, and L20), and other carcinoma lines (DON and ES-1) were established in UCLA surgical oncology. FB-7 and WHE were established from human fetal brain and whole human embryo, respectively. FL-1 is 2nd trimester fetal liver cells. HF-1 and -2 are fibroblast cells established in our laboratory. HT-29 is a colon carcinoma line. Molt-4 and CEM are T cell leukemias. Human cell lines were grown in RPMI 1640 (GIBCO) with 10% fetal calf serum, penicillin (100 units/m*l*) and streptomycin (100 mg/liter). S194 from BALB/c mice (10) is a non-immunoglobulin-producing, bromouridine deoxyribose (BudR)-resistant myeloma cell line which was kindly provided by the Salk Institute, San Diego, California.

Production of monoclonal antibodies

Two ten-week-old BALB/c mice were immunized by intraperitoneal injection with pulmonary squamous cell carcinoma cells (1×10^7) 2 weeks apart. Three days after last immunization, sensitized spleen cells (2×10^8 cells) were harvested, and fused with 4×10^7 S194 myeloma cells in 50% polyethyleneglycol 1,500 as described by Oi and Herzenberg (11). The fused cells were transferred to Costar 96-well microculture plates (6×10^5 cells/well) and cultured for 2 weeks in RPMI 1640 with 10% FCS containing hypoxanthine (13.6 mg/liter), aminopterin (0.176 mg/liter) and thymidine (3.88 mg/liter) (HAT medium) to select lymphocyte -myeloma hybrid cells. The selected hybrid cells were grown in culture medium containing hypoxanthine and thymidine (HT medium) for another week. Culture supernatants were tested against P3 cells and the autologous lymphoblastoid line PL3. Hybridomas showing more than five-fold preferential binding to P3 were selected for cloning by limiting dilution.

Antibody detection assay

An indirect protein A binding assay was used to detect hybridoma antibody binding to the P3 tumor cells as described previously (7): In brief, the target cells in suspension or as monolayers were incubated with hybridoma antibody, and incubated with rabbit anti-mouse immunoglobulins (DAKO 1 : 50), and then with ^{125}I-protein A (1×10^5 cpm/50μl). The proteinA reagent (SpA) was labeled with ^{125}I (2×10^7 cpm/μl SpA) by the vapor-phase chloramine-T method as described in (12). ^{125}I-bound target cells were counted by γ-counter.

Immunoprecipitation

Molecular weight of the antigens detected by monoclonal antibodies was determined by immunoprecipitation method. Five$\times10^7$ cells of P3 which was used for immunization to mice were labeled with ^{125}I by lactoperoxidase method. ^{125}I surface membrane labeled cells were kept at 0℃ for 20 min. with 1 ml of 0.5% NP-40. Then the mixture was centrifuged at 2,000×G for 15 min. and then further centrifuged at 25,000×G for 2 hrs. Supernatant was used for immunoprecipitation and two$\times10^7$ cpm of supernatant was mixed with monoclonal antibody for 12 hrs. at 4℃, then incubated with rabbit anti-mouse Ig conjugated with staphylococcus aureus (Sigma, Cowan I strain). Then the mixture was precipitated at 2,000×G for 20 min. The molecular weight of samples was determined by SDS-polyacrylamide gel electrophoresis using 10% acrylamide gel.

Competition assay

Monoclonal antibodies were preincubated with purified CEA from metastatic colon carcinoma, provided from the lst Dept. of Biochemistry, Hokkaido Univ. School of Med., Sapporo, Japan, at 4℃ overnight and then P3 target cells (2×10^5 cells/50 μl) were added. After 30 min. incubation and washing, rabbit anti-mouse Ig was added and then ^{125}I-protein A was also added.

RESULTS

Selection of monoclonal antibodies

After two weeks of HAT selection, culture supernatants from hybridoma primary culture wells were tested by using ^{125}I-protein A binding assay against P3 carcinoma cells and PL3 autologous lymphoblastoid cells in order to exclude HLA antigens. Hybridomas which produce antibodies showing more than five-fold preferential binding to P3 cells than PL3 cells were selected and cloned.

Three monoclonal antibodies reactive with carcinomas and embryonic cells

Three monoclonal antibodies 170C5, 754A3 and 806B4 from culture supernatants showed rather similar pattern of reactivity to a variety of tumor and non-tumor cells by ^{125}I-protein A binding assay. As shown in Table 1, 170C5 and 754A3 react to carcinomas, embryonic cells and some of melanomas but not to sarcomas and lymphoid cells. 170C5 and 754A3 show weak reactivity to human

Table 1 *Reactivity of monoclonal antibodies to cell lines*

		170C 5	754A 3	806B 4
Fetus and fibroblast				
	FB-7	+++	++	+
	WHE	++	+	++
	FL-1	+	+	+
	HF-1	±	±	−
	HF-2	±	−	−
Carcinoma	P1	+++	++++	+
	P3	+++	++	++
	P5	+	−	+
	P6	++	NT	+
	P7	++	−	++
	HT-29	+++	++	+
	DON	+	+++	++
	ES-1	NT*	+	++
Sarcoma	S1	−	−	−
	S3	−	−	−
Melanoma	M14	−	±	−
	M15	−	−	−
	M16	+	+	−
	M19	++	−	−
	M20	−	−	−
Lymphoid	PL3	−	−	−
	L14	−	−	−
	L20	−	−	−
	Molt-4	−	−	−
	CEM	−	−	−

*NT : not Tested

Table 2 *Immunoglobulin subclass of monoclonal antibodies*

Monoclonal Ab \ Ig class	Whole Ig	IgG_1	IgG_{2a}	IgG_{2b}	IgG_3	IgM
170C5(cpm)	10,321	862	7,369	1,421	798	820
754A3(cpm)	8,452	1,148	1,541	4,818	954	1,021
806B4(cpm)	7,085	1,043	4,563	1,242	852	1,243

P3 cells ($2\times10^5/50\ \mu l$) were incubated for 30 min. with hybridoma culture supernatant and incubated with class-specific rabbit anti-mouse immunoglobulin (1 : 50) and then incubated with ^{125}I-protein A.

fibroblast cells. 806B4 reacts to embryonic cells and carcinomas but not to sarcomas, melanomas, lymphoid cells and fibroblast cells.

Immunoglobulin class of monoclonal antibodies

Immunoglobulin subclass of these three monoclonal antibodies were also determined by an indirect three-step binding assay as shown in Table 2. P3 cells were used as target cells and incubated with each culture supernatant. Class -specific rabbit anti-mouse immunoglobulin was used as second antibody and then ^{125}I-protein A was added. As a result, 170C5 and 806B4 are IgG2a. 754A3 is IgG2b.

Antigen analysis

In order to determine molecular weights of protein detected by these antibodies, immunoprecipitation of the antibodies using ^{125}I-labeled membrane extract of P3 cells was performed and detect protein antigens by 10% SDS-PAGE as shown in Fig. 1. 170C5 and 806B4 precipitate 130K and 135K, respectively. 754A3 also precipitates 55K.

Blocking assay of two monoclonal antibodies

Two monoclonal antibodies 170C5 and 806B4 detect very close molecular weights and show the similar reactivity to tumor and non-tumor cells. So cross -blocking assay was performed whether the antigenic determinant detected by these antibodies is same or not. 170C5 and 806B4 were labeled with ^{125}I by vapor phase chloramine-T method. P3 cells were preincubated with 170C5 or 806B4 and ^{125}I-labeled 170C5 or 806B4 was added, respectively. ^{125}I-labeled 170C5 was blocked only by 170C5 not by 806B4 and ^{125}I-labeled 806B4 was blocked by 806B4 not by 170C5 (Table 3). So antigenic determinants detected by these two antibodies are different.

Competition assay using CEA

As these three monoclonal antibodies react with carcinomas and embryonic cells, competition assay was performed whether these antibodies can be competed by CEA or not. Ten or one μg in 50 μl of CEA was used for competition, and preincubated with each antibody ($\times$16 diluted) at 4℃ overnight. Then three step ^{125}I-binding assay was performed using P3 cells as target. As shown in Table 4, no competition of the antibody by CEA was observed.

DISCUSSION

In the present study, we analyzed three antigens detected by monoclonal antibodies from bybridomas produced against human squamous cell carcinoma P3. Monoclonal antibodies 170C5 and 754A3 react to carcinomas, embryonic cells and some of melanomas, and also react at a lower level to human fibroblast cells. 806B4 reacts to carcinomas and embryonic cells, not to sarcomas, melanomas and

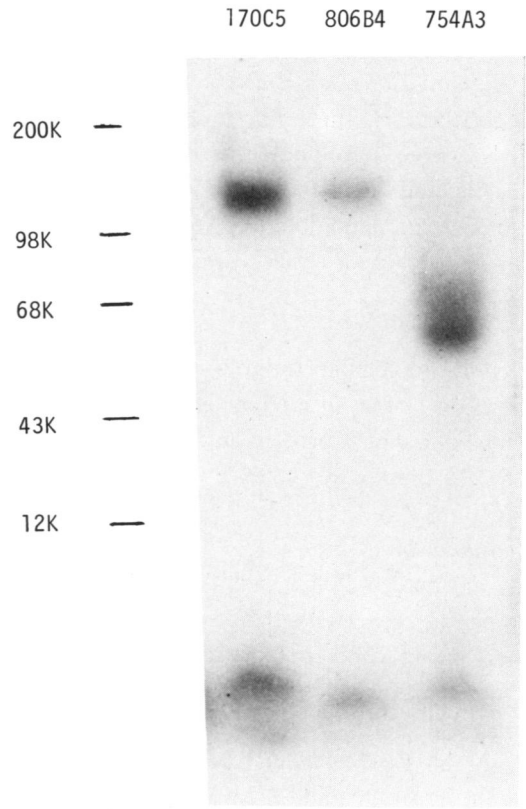

Fig. 1 ^{125}I-labeled membrane extract was immunoprecipitated by monoclonal antibodies 170C5, 754A3 and 806B4. Then precipitated proteins were run by SDS-PAGE using 10% acrylamide gel. 170C5 and 806B4 precipitate 130 K and 135 K, respectively. 754A3 also precipitates 55 K.

Table 3 *Cross blocking assay*

^{125}I-labelled Mo Ab / Preincubation with	170C5	806B4
170C5	384 cpm	5,186
806B4	4,215 cpm	287
NO	5,104 cpm	5,714

P3 cells were preincubated with MoAb 170C5 or MoAb 806B4 (undiluted) for 30 min. and then ^{125}I-labelled 170C5 or 806B4 (dilution 1 : 4) was added.

Table 4 *Competition assay using CEA*

Competition with \ Monoclonal antibody	170C5	754A3	806B4
None	4,284	3,677	4,042 (cpm)
CEA 10 $\mu g/50\,\mu l$	3,930	3,422	4,244 (cpm)
CEA 1 $\mu g/50\,\mu l$	4,024	3,468	4,188 (cpm)

Each monoclonal antibody (×16) was preincubated with 10 $\mu g/50\,\mu l$ or 1 $\mu g/50\,\mu l$ of CEA at 4℃ overnight and P3 target cells were incubated. Then rabbit anti-mouse Ig and ^{125}I-protein A were added.

lymphoid cells. By the protein analysis using immunoprecipitation and SDS -PAGE, monoclonal antibodies 170C5, 754A3 and 806B4 precipitated molecular weights of 130,000, 55,000 and 135,000, respectively. Each antigen is different from carcinoembryonic antigen which was reported to be a glycoprotein of 180,000 daltons (13) and these monoclonal antibodies are also different from those of Accolla's (14). And moreover, the binding of our three monoclonal antibodies to P3 cells were not inhibited by CEA proteins as shown in Table 4. Irie *et al.* have reported oncofetal antigen-I (OFA-I) which is distributed to 2nd trimester fetal brain and tumors of ectodermal and mesodermal origin (4). The distribution of OFA-I and three antigens detected by our three monoclonal antibodies are different and the characterization of OFA-I_2 is ganglioside (15), while our antibodies detected protein antigens.

In the process of analyzing the antigens expressed on human lung cancers, three monoclonal antibodies detected protein antigens which would share the part of oncofetal antigens. Furthermore, precise analysis of many other oncofetal antigens must be done in order to distinguish tumor associated antigens from oncofetal antigens.

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