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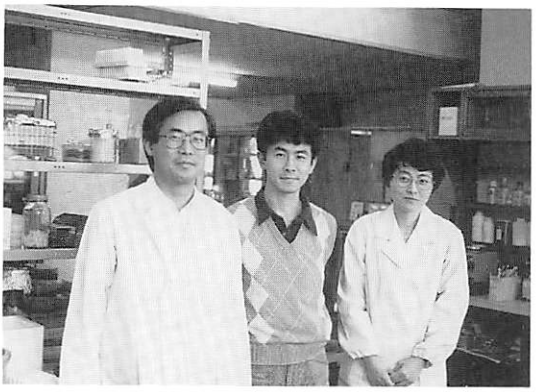
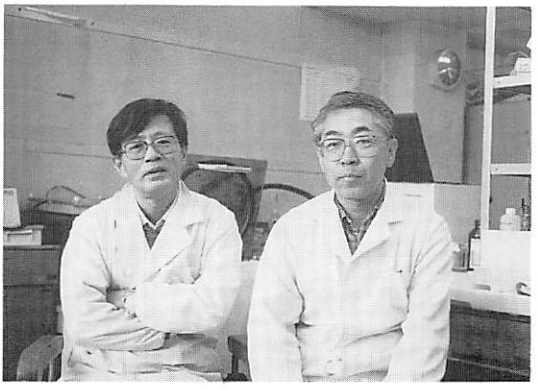
Pathophysiology





DEPARTMENT OF PATHOPHYSIOLOGY

GENERAL SUMMARY

Identification, purification and characterization of antigens specific to or associated with human tumors as particular tumor markers are one of the most important problems in the field of tumor immunology. Through the efforts of a number of investigator to detect the tumor-specificity in assays for humoral and cellular immunity, it has been proven that specific antigens expressed only on tumor cells exist. Previous work in our laboratory has shown that the antigens specific to or highly associated with tumors could be detected in various human cancers and rat ascites hepatomas by gel diffusion, immunofluorescence and enzyme-linked immunosorbent assay (ELISA) using membrane fractions of tumors and extensively absorbed hyperimmuned polyclonal antibodies. For the past three years, we have carried out research in the following four fields.

(I) Establishment of purification methods of tumor antigen.

The tumor specific or highly associated antigens solubilized from the insoluble membrane fraction of a variety of human and rat carcinomas with the aid of sodium deoxycholate (DOC) were partially purified by Sepharose 4B gel, Blue Sepharose CL-6B gel, ion-exchange gel and affinity chromatography. The antigens were further purified by zone electrophoresis on agarose gel and repeated HPLC. These purified antigens were identified by the method of immunoprecipitation, immunoblotting (Western blotting) and two dimensional gel electrophoresis in addition to gel diffusion, ELISA and immunofluorescence. These antigens were generally glycoproteins restricted on cell membrane and had molecular weights of about 40,000 to 70,000 daltons, $\beta_2-\gamma$ mobilities and pI of 4.8 to 5.3.

(2) Function of tumor antigens.

We have found that some of these tumor antigens have EGF/TGF and/or Insulin receptor function. But their molecular size were different from that of known FGF/TGF-receptor (Mr. 170Kd) or Insulin-receptor (Mr. 460Kd). Furthermore, they showed tyrosine kinase activity on autophosphorylation.

(3) Preparation of monoclonal antibodies against tumor antigen. Several kinds of monoclonal antibodies against human thyroid carcinoma, renal cell carcinoma or stomach adenocarcinoma were prepared. Characteristics of tumor antigens recognized by these antibodies were examined on application of cell-ELISA, immunoblotting and immunohistochemical stainding methods.

(4) Isolation of gene cording tumor antigen and E. coli production of the antigen with the gene.

cDNA library for human thyroid carcinoma was screened with monoclonal antibody against thyroid carcinoma. About one-third of the total DNA cording thyroid tumor antigen was isolated. With the isolated gene, the antigen fragment reactive to the antibody was produced in E. coli, and separated purely in affinity chromatography.

(1) Human carcinoma-associated or -specific antigens capable of binding epidermal growth factor, transforming growth factor and/or insulin with tyrosine-specific phosphokinase activity.

S. Okada.

The receptor of epidermal growth factor (EGF), transforming growth factor-α (TGF-α), insulin and insulin-like growth factor-I (IGF-I) and the retroviral oncogene (src family) products have been shown to possess important roles as substances having tyrosine-specific protein kinase activity in tumor proliferation and cell transformation.

We have found that some of the tumor-associated or -specific glycoprotein antigens purified from the variety of human and murine carcinoma have a EGF- or TGF-receptor function. But their molecular size were different from that of known EGF/TGF-receptor (Mr. 170Kd) or Insulin-receptor (Mr. 460Kd). Moreover, these antigens showed tyrosine-specific phosphokinase activity on binding with these three growth factors.

In human renal cell carcinoma (RCC), when the RCC-associated antigen ($gp68^{rcc}$) was incubated with TGF or EGF and insulin, it was reactive with monoclonal antibodies to these three growth factors on ELISA and immunoblotting using SDS-PAGE at a single band of 68Kd. The positive reaction of ^{125}I-EGF and ^{125}I-insulin to $gp68^{rcc}$ was also shown by autoradiography on SDS-PAGE (Fig. 1). Autophosphorylation of $gp68^{rcc}$ with γ-^{32}P-ATP was recognized in the presence of EGF or insulin, and tyrosine was specifically phosphorylated (Fig. 2). These results suggest that our tumor-associated or -specific antigens may be some of the oncogene products related to the src family and may be the membrane bound glycoprotein receptors related to tumor proliferation and cell transformation.

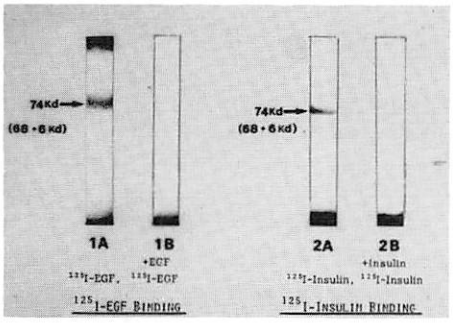


Fig. 1. Autoradiography of ^{125}I-EGF and ^{125}I-insulin binding reactions to $gp68^{rcc}$ in absence and presence of unlabelled EGF and insulin on 10% SDS-PAGE.

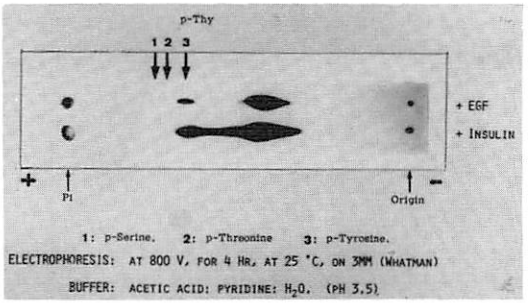


Fig. 2. Autoradiography for identification of phosphotyrosine in $gp68^{rcc}$ on high voltage paper electrophoresis.

(2) A tumor-specific antigen in human Wilms' tumor.

S. Okada and H. Yamamoto

The isoluble franction of human Wilms' tumor (nephroblastoma) tissue was solubilized with the aid of deoxcholate. The solbilized fraction, after partial purification, was used to produce the antisera in guinea pigs. A selected antiserum, absorbed extensively with normal human serum and homogenates of fetal kidney, adult kidney, liver, spleen, lung, and colon carcinoma, was used. The antiserum was reactive on ELISA with all 4 Wilms' tumor tested, but not with fetal kidney, normal adult organs and other malignant neoplasms.

Immunofluorescence studies showed that the antigen was membrane-associated. The antigen showed $\beta_2 \sim \gamma$-mobility on zone electrophoresis in agarose gel combined with ELISA. When the compornents of $\beta_2 \sim \gamma$-mobility were extracted and submitted to Western blotting, a single compornent of 58,000 dalton was immunostained with the absorbed antiserum by the ABC staining method. The purified antigen by HPLC was thought to be a glycoprotein which contained about 7% glycoside because the antigen treated with endoglycosidase H showed a molecular weight decrease to 54,000 dalton on SDS-PAGE When 58 Kd antigen was incubated with ^{125}I-EGF, treated with disuccinimidyl suberate, and submitted to SDS-PAGE, a single compornent of apparent molecular weight of 64 Kd (58Kd+6Kd) was detected by autoradiography.

This labelling was virtually blocked under the condition of an excess amount of unlabelled EGF in the binding medium. These results suggest that a tumor-specific or associated EGF receptor of 58Kd exists in Wilms' tumor.

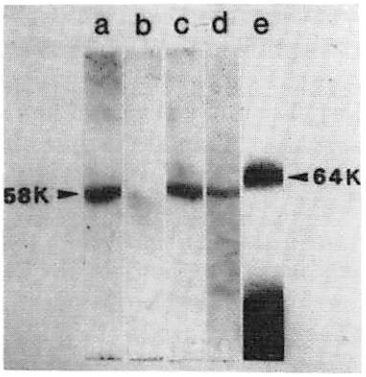


Fig. 1. Immunoblotting of the 58Kd-blot of Wilms' tumor with peptide hormones and Autoradiography.
Lane a, staining with anti-Wilms' tumor antiserum (α-W);
Lane b, incubation with EGF followed by staining with α-W;
Lane c, incubation with insulin followed by staining with α-W;
Lane d, incubation with EGF followed by staining with anti-EGF monoclonal antibody;
Lane e, formation of the complex with Mr 64,000 by incubation of the antigens with ^{125}I-EGF (Autoradiography).

(3) Purification and characterization of a tumor-specific surface antigen in murine ascites hepatoma AH7974

S. Okada, K. Takeyama amd Y. Kurata

The insoluble extract of rat ascites hepatoma AH7974 cells was solubilized with the aid of deoxycholate, and the solubilized proteins (SP) were used to produce polyclonal antisera in guinea pigs. A selected antiserum, after suitable absortion, was proven to detect a surface antigen specific to AH7974 by immunodiffusion, living cell membrane immunofluorescence and enzyme-linked immunosorbent assay (ELISA). A tumor-specific glyco-protein antigen was purified from the SP of AH7974 by high performace liquid chromatography (HPLC).

The antigen was shown to have β_2-mobility in zone electro-phoresis in agarose gel and an apparent molecular weight of 58,000 daltons in SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

It was capable of specifically binding epidermal growth factor (EGF). (1). AH7974 SP showed a single specific immuno-diffusion with absorbed anti-AH7974 serum. AH7974 fraction also had high antigen activity on ELISA, but SP fractions from control tissues and cells had negligible activities. Living cell membrane immunofluorescence techniques demonsteated that a specific antigen was restricted in the cell surface membrane of AH7974 cells, but not in the membrane of other ascites hepatoma cells. (2). The specific antigen had EGF receptor activity, caused covalent binding to EGF or ^{125}I-EGF, and produced a complex binding molecule of 64 Kd (AH7974 gp58 Kd+EGF 6Kd).

When AH7974 living cells were used, these 64 Kd molecules were also produced in the binding experiments with ^{125}I-EGF.

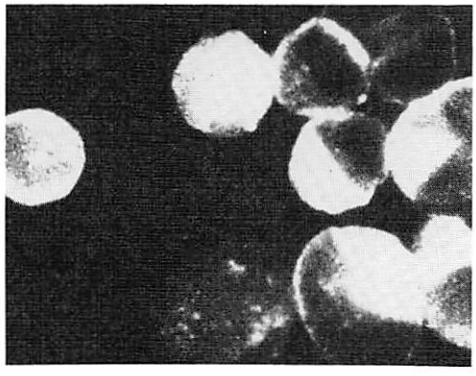


Fig. 1. Living cell membrane immunofluorescence with absorbed anti-AH7974 antiserum.
The antigen appear to be located on the plasma membranes of AH7974 cells. Uniform staining (smooth ring).

(4) An antigen highly associated with human gastric adenocarcinoma.

M. Seki, S. Okada and Y. Kurata

In studies concerning human gastric carcinoma, a number of antigens have been detected, but antigens specific to or highly associated with tumors have not yet been found. The isoluble fraction of gastric adenocarcinoma was solubilized with the aid of deoxycholate. Then the solubilized ptotein (SP) was passed through a column of Sepharose 4B and Blue Sephareose CL-6B to remove contaminated ingredients. This fraction was used to produce hyperimmune antisera in guinea pigs. A potent antiserum αSC5 was selected and absorbed extensively with human whole serum and organ homogenates. The absorbed antiserum was used throughout the experiments for ELISA and immune blotting. The SPs from gastric adenocarcinomas and a gastric adenocarcinoma cell line MKN-28 passed through Blue Sepharose DL-6B were analyzed on HPLC and ELISA with absorbed αSC5, showing an active peak (P2) eluting close to the albumin position's tail. The SPs from normal adult organs, a fetal stomach, and a fetal gut showed no P2 activity. Agarose gel electrophoresis showed that the P2 components from gastric adenocarcinomas and MKN-28 have β_2-mobility, while the components at the P2 region of normal adult and fetal organs have only albumin$\sim\beta_1$-mobility.

Western blotting of the α_2-components from gastric adenocarcinoma and MKN-28 showed a putative tumor-specific 59Kd band. When examined by a H-LAI test using the eluate from the β_2-region of a gastric adenocarcinoma, positive responses of sera from patients with gastric adenocarcinoma and negative responses of control sera were observed.

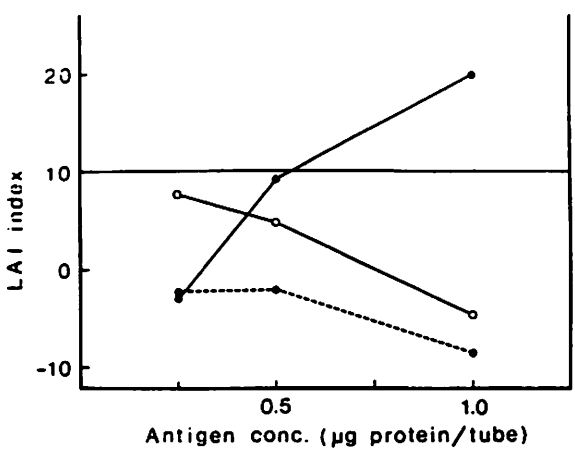


Fig. 1. Effect of cocentration of eluates at the β_2-region on agarose gel electrophoresis of solubilized proteins from a gastric adenocarcinoma (•) and a lung carcinoma (○) on a humoral leukocyte adherance inhibition test of sera from a patient with gastric adenocarcinoma (———) and from a control person (-----).

(5) Monoclonal antibodies against human renal cell carcinoma.

H. Shimizu and S. Okada

BALB/c mice were immunized with the deoxycholate solubilized fractions from the insoluble extracts of human renal cell carcinoma (RCC). Spleen cells were fused with SP2/0 myeloma. Hybridomas which were found positive on human RCC cells and negative on human embryonic kidney epithelium were selected and cloned. Monoclonal antibodies (MABs) designated as KRC-5 and KRC-6 are of the IgG1 subclass. MABs designated as KRC-9 and KRC-10 are of the IgM subclass.

The specificity of each MAB was examined. The reactivities of MABs against various human cell lines in Cell-ELISA are listed in Table 1. The results of the immunohistochemical staining of MABs on human tumor and normal tissues are shown in Table 2. KRC-5 stained 8 of 8 RCCs, but not normal kidneys. KRC-5 reacted with some cell lines other than RCC. KRC-6 stained 8 of 8 RCCs and the proximal tubules of normal kidneys. However, KRC-6 didn't stain any other tissues. KRC-9 and KRC-10 stained 2 of 8 RCCs, but not normal kidneys. Four MABs all stained membranes of RCC tumor cells.

Preliminary studies were performed on the characterization of antigens. KRC-5 recognizes carbohydrates containing sialic acids, because the determinant detected by KRC-5 lost its activity upon treatment with neuraminidase. The determinant detected by KRC-6 is heat labile, so it is probably a peptide. The other two antigenic systems detected by KRC-9 and KRC-10 are defined as glycolipids, because of heat stability and solubility in organic solvents.

Table 1. Reactivity of MABs against various human cell lines in Cell-ELISA.

Cell line	Origin	KRC-5	KRC-6	KRC-9	KRC-10
ACHN	RCC	++	+	+	+
NCC75	RCC	+	++	+	±
NRC-12	RCC	+	++	-	-
Ko-RCC-1	RCC	-	++	-	-
MKN-45	Stomach Ca.	-	-	++	+
CHC-4	Hepatoma	++	-	-	-
ZR-75-1	Breast Ca.	++	-	-	-
IMR-32	Neuroblastoma	++	-	-	-
OST	Osteosarcoma	+	-	-	-

Cell lines listed below were nagative with four MABs. RCC (KU-2,KN41), Bladder Ca. (T-24, KK47), Stomach Ca. (MKN-1, KATO-III), Colon Ca. (COLO201), Pancreas Ca. (PANC-1, MIAPaCa-2), Lung Ca. (PC-6), Uterus Ca. (HeLa), Ovary Ca. (HOC-21), Larynx Ca. (HEp-2), Wilms' tumor (G401), Rhabdomyosarcoma (A204), B-cell type ALL (BALL-1), T-cell type ALL (CCRF-CEM), Fetal intestine (Intestine407).

Table 2. Reactivity of MABs with human tumor and normal tissues. Immunohistochemical staining was performed using the ABC method on cryostat sections after fixation in absolute ethanol.

Tissue	No. of cases tested	No. of positive cases			
		KRC-5	KRC-6	KRC-9	KRC-10
RCC	8	8	8	2	2
Stomach Ca.	2	0	0	0	0
Colon Ca.	1	0	0	1	1
Kidney	3	0	3	0	0
Thyroid	2	2	0	0/1	0/1
Lung	2	0	0	0/1	0/1

Normal tissues listed below were 0/1 with four MABs. Stomach, liver, spleen, pancreas, small intestine, large intestine.

(6) Monoclonal antibodies against human thyroid cancer

N. Satoh and Y. Kurata.

The cell line of thyroid papillary adenocarcinoma (PC-1) was established as a source of antigen. The cell line excreted thyroglobulin, as a spesific marker, into the medium at 3.7ng per ml.

Antigen was prepared from the membrane fraction of TPC-1 which was solubilized with the aid of deoxycholate followed by cold acetone precipitation and Blue Sepharose CL-6B gelfiltration. Monoclonal antibodies against thyroid carcinoma have been produced by the fusion of mouse myeloma cells (SP2/0) with splenic lymphosytes of BALB/c mice after 5 times immunization each with Img of antigen. Using screening by cell ELISA, monoclonal antibodies KTC-3 (IgM) and KTC-4 (IgM) were selected. These antibodies were nonreactive to normal thyroid follicular cells but were reactive to thyroid papillary carcinoma and anaplastic carcinoma. Cell lines from seventeen other tumors were nonreactive except for a few cases (Table.1). By living cell membrane limmunofluorescence technique and the Avidin-Biotin staining method, the antigen was detected on the cell membrane (Fig. 1). The epitope recognized by these monoclonal antibodies was sensitive to Pronase E and insensitive to Meta periodic acid, and was regarded as a peptide. In Western blot analysis of antigen resolved by one-dimensional electrophoresis, the monoclonal antibodies recognized a specific component with an apparent molecular weight of 6,1500 (Fig.2).

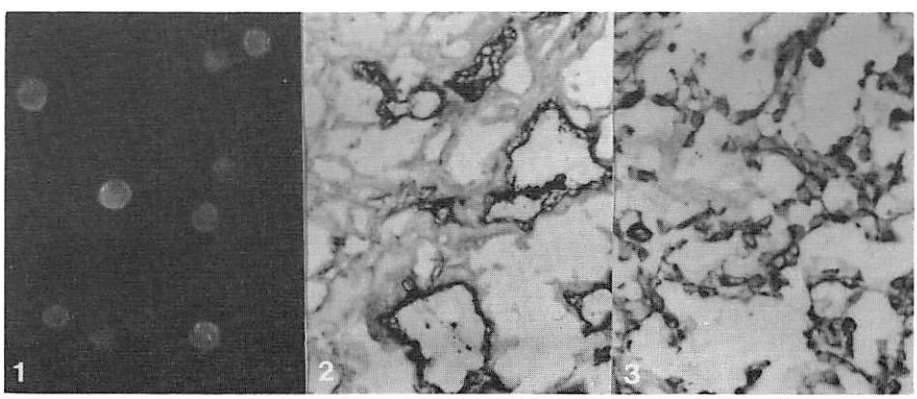


Fig. 1. Detection of Surface Antigen.
1. Living membrane immunofluorescence of TPC-1 cells with antibody KTC-3.
2. Avidin-Biotin staining of a frozen section of papillary carcinoma with antibody KTC-3.
3. Avidin-Biotin staining of a frozed section of anaplastic carcinoma with antibody KTC-3

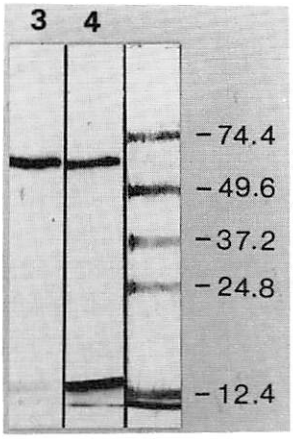


Fig. 2. Western blot analysis of antigen.
Lane 3; With monoclonal antibody KTC-3.
Lane 4; With monoclonal antibody KTC-4.

Table 1. Reactivity of monoclonal antibody KTC-3 with various cell lines by cell ELISA

Cell line	Origine	Reactivity
(Normal cell)		
NTC-I	Normal thyroid cell	–
HEK	Human embryo Kidney	–
Intestine 407	Human embryo intestine	–
Bec II	Lymphocytes of tonsillitis	–
CCD-33co	Human colon fibroblast	–
(Thyroid cancer)		
TPC-I	Thyroid papillary carcinoma	+
TPC-2	〃	+
TUC-I	Thyroid anaplastic carcinoma	+
THC-4-JCK	Thyroid papillary carcinoma	+
THC-5-JCK	Thyroid anaplastic carcinoma	+
(Other tumors)		
1). MKN-I	Carcinoma of the Stomach	–
MKN-28	〃	–
MKN-45	〃	+
Kato III	〃	–
2). COLO 20I	Carcinoma of the Colon	–
3). Panc-I	Carcinoma of the Pancleas	–
Mia Paca-2	〃	–
4). CHC-4	Carcinoma of the Liver	–
5). Hep-2.	Carcinoma of the Larynx	–
6). PC3	Carcinoma of the Lung	–
PC6	Carcinoma of the Lung	–
PC10	〃	–
7). Hela	Carcinoma of the Uterus	–
8). HOC	Carcinoma of the Ovary	–
9). ZR-75-1	Carcinoma of the Brest	–
10). G401	Wilms' Tumor	–
Tuwi	〃	–
11). RCC KN41	Carcinoma of the Kidney	+
Ku-2	〃	+
NRC-12	〃	–
NCC	〃	–
KoRCC	〃	–
12). T24	Carcinoma of the Bladder	–
13). HMV	Melanoma	–
14). IMR32	Neuloblastoma	–
15). Ast	Astrocytoma	–
16). OST	Osteo sarcoma	–
MG63	〃	–
17). TALL-1	T-ALL*	–
CCRF-CEM	〃	–
Daudi	African Burkitt's lymphoma	–
HL-60	Acute promyelocytic leukemia	–
K562	Chronic myelogenous leukemia	–

Abbreviation: T-ALL, T-cell type acute lymphoblastic leukemia.

(7) Isolation of gene coding human thyroid carcinoma associated antigen and production of the antigen in E. coli

N. Satoh, S. Sugiura[1], K. Yamaguchi*, T. Kameyama* and Y. Kurata

Human thyroid carcinoma cDNA library in expression vector λgtll (lac5 cI_{857} nin5 S100) was screened on the lawn of E. coli Y1090 (supF) with monoclonal antibody KTC-3 recognizing thyroid carcinoma associated antigen with an apparent molecular weight of 6,1500 (see previous abstract).

The site used for insertion of thyroid carcinoma DNA is a unique EcoRI cleavage site located within the lacz gene, 53 base pairs upstream from the β-galactosidase translation termination codon. In the Western blot and Peroxdase anti Peroxdase (PAP) staining method, positive signals were detected at a rate of one per 500-1000 plaques.

A recombinant phage clone λgtll (TAA^+) with a positive signal was purified, and the lysogen of E. coli Y1089 (HflA 150) with λgt11 (TAA^+) was constructed. In the presence of IPTG, an inducer, the lysogen produced a fusion protein with β-galactosidase and peptide reactive to monoclonal antibody KTC-3 (Fig. 1).

λgtll (TAA^+) was harvested from E. coli Y1089 [λgtll (TAA^+)] and a DNA insertion 600 base pairs in size was cleaved with EcoRI from the cloning site of λgtll (Fig. 2). The size of 600 base pairs corresponds to about one-third of a gene cording tumor associated antigen of human thyroid carcinoma.

600bpDNA was connected, in frame, to the downstream of the cording sequence for Staphylococcal Protein A at the Sma I site in a vector $_pRIT2T$ through the EcoRI-SmaI adaptor (Fig. 3).

$_pRIT2T$ protein A fusion vector systems offer regulatory advantage when grown in a host strain containing the λcI_{857} temperature sensitive repressor, and also offer the performance of specific chemical cleavage, cleaving the fusion protein between Protein A and the peptide.

Recombinant vector clone $_pRIT2T$ (TAA^+) in E. coli N4830-1, containing λcI_{857} temperature sensitive repressor, produced fusion protein with Protein A and the peptide by shifting the temperature from 30°C to 42°C. The cell lysate containing fusion protein with Protein A and the peptide was applied to an IgG Sepharose 6Fast Flow affinity column containing the specific IgG ligand. After elution, performed by lowering the pH, the purified fusion protein was collected in the effluent. The purified fusion protein was treated with 70% formic acid in order to cleave acid labile Asp-Pro peptide bond intoduced, by site directed mutagenesis, at the fusion point between protein A and the peptide. The mixture was again passed through the column, the peptide reactive to antibody KTC-3 was collected in the effluent, while Protein A was absorbed to IgG ligand and was eluted by lowering the pH (Fig. 4).

1) Institute for Gene Research, Kanazawa University

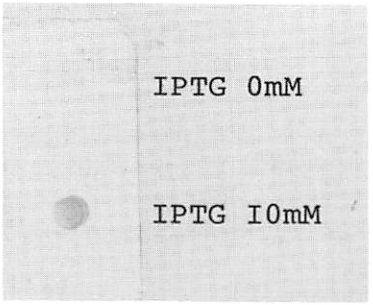
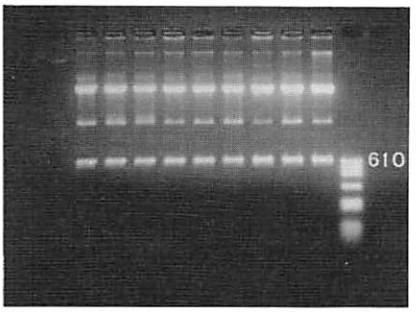


Fig. 1. The lysogen bacteria was induced with IPTG. Cell lysate showed reactivity to monoclonal antibody KTC-3.



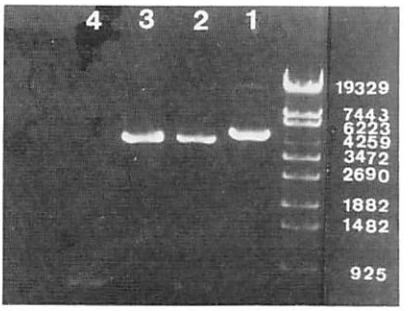


Fig. 2. 600bp DNA fragment was obtained by digestion with EcoRI of DNA of recombinant λgtll harvested from lysogenic bacteria E. coli Y1089. The DNA fragment was cloned into the EcoRI site of plasmid PUC 19, which transformed E. coli JM109. From 9 colonies of transformant, plasmid DNA was extracted. 600bp of the DNA fragment was obtained by EcoRI digestion.

Fig. 3. The DNA fragment was connected to the downstream of Protein A resion in plasmid $_{p}$RIT2T.

Lane 1; $_{p}$RIT2T DNA+DNA fragment.

Lane 2; $_{p}$RIT2T DNA and DNA fragment.

Lane 3; $_{p}$RIT2T DNA.

Lane 4; DNA fragment.

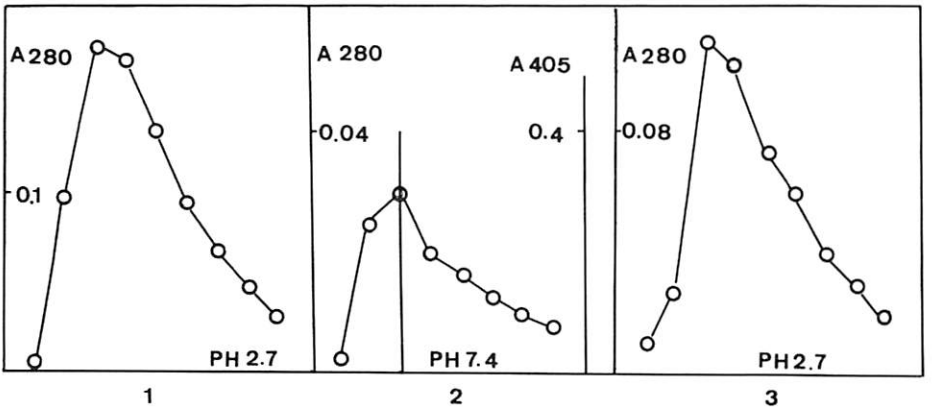


Fig. 4. Separation of Protein A and antigen with IgG Sepharose 6 Fast Flow affinity chromatography.

1. Fused protein.
2. Antigen.
3. Protein A.

(8) Mouse monoclonal antibodies with restricted specificity for human renal cell carcinoma and ability to modulate the tumor cell growth in vitro.[1)]

H. Tokuyama[2)] and Y. Tokuyama

Many monoclonal antibodies (MonAbs) against human renal cell carcinoma (RCC) have been reported: some of them are highly specific to RCC and others define differentiation antigens of renal cells or virus-related antigens. However, biological functions of the RCC-associated antigens are poorly understood.

We produced three mouse MonAbs (KRC-1, -2 and -3) against human RCC cultured cells (Ku-2 and KN41). All the three MonAbs reacted with only two RCC cell lines (Ku-2 and KN41) of six RCC cell lines tested, as examined by cell-ELISA. They showed no reactivity to 37 other cell lines of various types of human tumor, peripheral blood lymphocytes, erythrocytes, embryonal kidney cells or normal thyroid cells. KRC-1 and -3 stained only a part of RCC tissues tested, 3/8 and 2/8, respectively. However, KRC-2 showed no binding to the 8 RCC tissues tested. None of the three stained normal tissues, including kidney. Therefore, specificity of the three MonAbs seems to be highly restricted to the subset of RCC.

The effect of the three MonAbs on growth of Ku-2 cells was examined in an anchorage dependent culture system. All the three MonAbs of IgGl subclass showed modulation effects, that is, KRC-1 stimulated but KRC-2 and -3 suppressed the tumor cell growth. The ratio of cell number in the experimental culture with MonAbs ascitic fluid (1:200-1:500 dilution) to that in the control culture without MonAbs was about 1.5 with KRC-1 and 0.4-0.5 with KRC-2 and -3. Similar modulation effects were observed by colony formation assay in soft agar, too. The cell line specificity was confirmed in the modulation effects.

KRC-1 detected a 135Kd glycoprotein in immunoblotting. KRC-2 detected a high molecular weight antigen (Ca. 1,200kd) in Sephacryl S 300 gel-filtration. 170Kd and 83Kd polypeptides were revealed by KRC-3 affinity purification. All the antigens showed a high affinity for Con A lectin. The stability test by trypsin and Pronase E, the lectin affinity and chloroform-methanol extraction test suggested that KRC-1 and -3 are glycoproteins and that KRC-2 is of carbohydrate nature. The molecular properties of the three antigens appear to be different from those of previously reported RCC-associated antigens. The three MonAbs seem to be useful for studying the mechanism of growth regulation in transformed cells.

1) H. Tokuyama, Y. Tokuyama and Y. Kurata.: Proc. Jpn. Cancer Assoc., 44th. Ann. Meet. (Tokyo), 137 (1985). H. Shimizu, H. Tokuyama, Y. Tokuyama and Y. Kurata.: ibid, 45th. Ann. Meet. (Sapporo), 133 (1986).

2) Dept. of Mol. Immunol.

(9) Two distinct epidermal growth factor-dependent transforming growth factors isolated from bovine colostrum.

H. Tokuyama[1] and Y. Tokuyama

The well-defined TGFs are classified into two types, TGF-α and TGF-β. TGF-α enables untransformed cells such as NRK-49F cells to form a colony in soft agar. TGF-β absolutely requires EGF or TGF-α in colony formation. Aquisition of anchorage-independent cell growth is shown to be a cellular property in vitro well correlated with neoplastic growth in vivo. However, the physiological role of TGFs has not been extensively studied.

We have tried to isolate TGFs from bovine colostrum. Two distinct epidermal growth factor (EGF)-dependent TGFs (21K and 7K TGF) have been partially purified from bovine colostrum. The 21K TGF is a major one and has been isolated by a sequence of acid-decaseination, DEAE-sephacel chromatography and acid gel filtration. The purification was monitored by a simplified colony formation assay in soft agar using normal rat kidney fibroblast cells (NRK-49F). Its apparent molecular weight is 21,000 daltons by acid gel filtration. The 21K TGF showed colony formation activity only in the presence of EGF. When the decaseinated colostrum was extracted by an acid-ethanol procedure, followed by the ion-exchange chromatography and acid-gel filtration, only a low molecular weight TGF (about 7,000 dalton) was found and the 21K TGF was no longer detected. The 7K TGF also required EGF for the activity. Both the 21K and 7K TGFs were resistant to heat (100°C 3 min) and were destroyed by trypsin. However, they were different in sensitivity to reducing agents, the 21K TGF was sensitive and 7K TGF resistant. Chemical properties of the 21K TGF seem to be different from those of the well-defined TGF-β that has a molecular weight of 25,000 daltons and is stable in acid ethanol extraction. The 7K TGF is also distinct from the known TGFs in that it is resistant to reducing agents.

In our preliminary experiment, the crude bovine colostrum and decaseinated colostrum inhibited the growth of some tumor cell lines and showed no effect on untransformed cell lines in anchorage dependent culture. Further purfication and characterization of these TGFs and the inhibitory activity are needed to elucidate correlation between the TGFs and the inhibitory activity.

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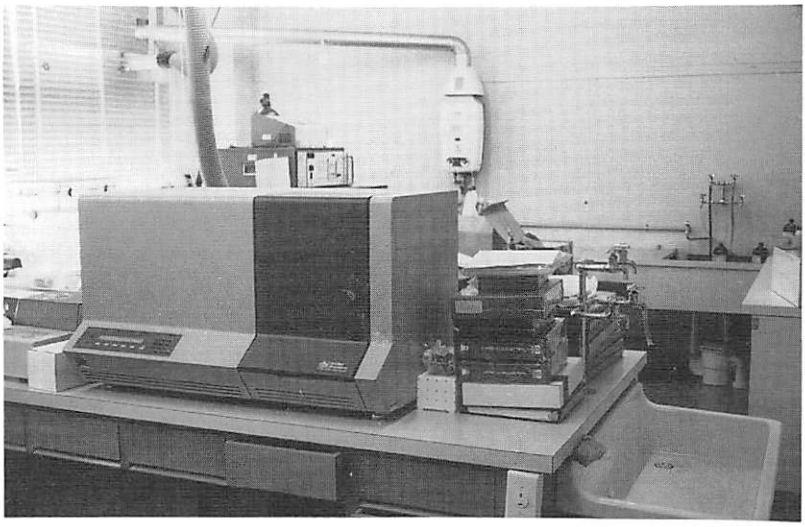
General Summary (addendum)

After the retirement of Dr.Yoriaki Kurata, Dr. Ken-ichi Yamamoto, formerly Associate Professor of the Department of Molecular Immunology, was appointed Head of the Department of Pathophysiology at the end of 1987. Dr. Yamamoto has been studing the molecular biology of acute phase reaction for past four years. He determined the genomic structure for mouse serum amyloid A proteins (SAA) which are most prominent acute phase reactants. In addition, he established that the induction of SAA gene expression during acute phase reaction is mediated by macrophage-derived cytokines such as interleukin-1 (IL-1), tumor necrosis factor (TNFα), and B-cell stimulatory factor 2 (BSF2: also called β2-interferon or IL-6). Thus, the major attention of this department under the new head will focus on molecular mechanisms which regulate the expression of genes involved in acute phase reaction (SAA, IL-1, TNFα, BSF2 and metallothionein), though the projects (the characterization of tumor associated antigens) studied under the direction of Dr. Kurata remain to be pursued.

1. SAA, a major component of amyloid deposits found in tissues of patients with secondary amyloidosis, is a most prominent acute phase reactant. We are interested in the molecular mechanisms of SAA gene regulation during acute phase reaction. Although IL-1 has been considered to be sole mediator of acute phase reaction, we found that other macrophase-derived cytokines, TNFα and BSF-2, also have the ability to provoke acute phase reaction. We are currently working on the induction by these cytokines of SAA gene expression in the transient gene expression system using human hepatoma cell lines such as HepG2 and Hep3B. We wish to determine the promotor structure of SAA genes involved in gene activation by these cytokines and to identify and characterize trans-acting factors which are activated by cytokines and then interact with the promotor region of SAA genes.

2. TNFα, one of cytokines secreted by activated macrophase, is a multi-potential cytokine and play a crucial role in the host reaction against tumor. TNFα is cytostatic to certain types of tumor cell lines, but induces cell growth of some cell lines such as diploid fibroblast and astrocytomas. TNFα is also a mediator of cachexia associated with cancer. We wish to study how TNFα mediates these diverse activities. We are particularily interested in the molecular mechanisms of inhibition or induction by TNFα of cell growth. We are currently working to identify genes activated by TNFα in TNF-sensitive or resistant cell lines. The preliminary experiments show that some c-oncogenes and cytokine genes are activated by TNFα. We hope that our study leads to better understanding of molecular mechanisms of cell growth regulation.



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