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DEPARTMENT OF PATHOPHYSIOLOGY

GENERAL SUMMARY

Tumor-specific antigens (TSA) will certainly play an important role in the diagnosis and treatment of the cancer diseases. The presence on human tumor cells of antigens not shared by their normal counterparts is being increasingly reported. Generally, however, it has been difficult to demonstrate the presence of any truly tumor-specific antigens in different kinds of human malignancy. In the area where tumor-specificity is being investigated, the inability to obtain the general method applicable to all tumors for immunochemical fractionation leading to antigen enrichment has been an acute problem. We have therefore embarked on a major effort in this direction.

We already demonstrated that the fractions (designated as LPfr2) prepared from the cellular insoluble lipoprotein fractions of thyroid gland, salivary glands, adrenal gland, thymus, muscle, testis, and liver by solubilization with desoxycholate and by gel filtration on Sepharose 4B contained the organ-specific antigen with several common antigens which were shared by different organs. The organ-specific antigenic fraction could be isolated by submitting the LPfr2 preparation to affinity chromatography on the Sepharose 4B which linked with the globulin of the potent xenoantisera for the common non-specific antigens.

Using similar techniques, we have aimed at identifying and characterizing the TSA in a variety of human neoplasms. The LPfr2 (lately designated as LPfr) prepared from human nephroblastomas, transitional cell carcinomas, adenocarcinomas and squamous cell carcinomas of the lung, pancreatic adenocarcinomas, gastric cancers etc. were used to raise antisera in guinea pigs. Unabsorbed and variously absorbed antisera were analyzed by double immunodiffusion. The antisera, after suitable absorption, were reactive with LPfr of corresponding tumors, but failed to react with LPfr of normal adult organs and fetal organ homogenates as well as LPfr of tumors of different histologic types. The antigens were shown distinct from carcinoembryonic antigen, alpha-fetoprotein, histocompatibility antigens, and blood group antigens. The electrophoretic mobilities of the antigens ranged from β_1 to near γ with tumors. The approximate molecular weights of some purified TSA were determined. The antigens contained carbohydrate (a few percent of protein) and virtually no lipid. Enzymic degradation studies showed that the protein part was of major importance to the integrity of the antigens. Immunofluorescence studies suggested that these TSA were localized in the cell membranes of tumor cells.

Immune RNA extracted from the xenogeneic animals immunized with LPfr of human gastric cancers (immune-RNA recognizing TSA of gastric cancer) has been efficaciously used in immunotherapy of a patient with cancer. Further examination of the effect is under way.

ABSTRACT

(38) Identification of a tumor-specific antigen in the insoluble fraction of human nephroblastoma

S. Okada, K. Itaya and Y. Kurata

The cellular insoluble lipoprotein fraction of nephroblastomas was solubilized by desoxycholate and the soluble fraction was submitted to column chromatography on Sepharose 4B (Fig. 1). The included fractions, called LPfr, were pooled and concentrated by pervaporation and used as antigen.

Guinea pigs were injected three times with LPfr of a nephroblastoma containing 10 mg protein over a one month period. The antisera obtained were oligospecific. They gave a single distinct precipitin line with LPfr of nephroblastomas and sometimes faint lines with LPfr of the liver and heart but failed to give a precipitin line with LPfr of other adult organs (Fig. 2) and fetal organs tested. Tests with the individual LPfr of a variety of human tumors revealed that the reactive antigen appeared to be restricted to the nephroblastoma LPfr. On immunoelectrophoresis the antigen showed a mobility slightly faster than IgG (Fig. 3) while the normal kidney antigen showed α_1-mobility.

The nephroblastoma LPfr appeared to be nearly homogeneous as judged by immunodiffusion, but it showed several faint bands plus one prominent band on disc electrophoregram. By column fractionation in the presence of 6 M guanidine hydrochloride, it separated into at least four components whose molecular weights were about 100,000, 72,000, 60,000, and 47,000 D. Experiments on the fraction purified by reverse immunoabsorption suggested that the specific antigen was the component of about 60,000 D.

The presence of the antigen in sera of three patients with nephroblastoma was observed.

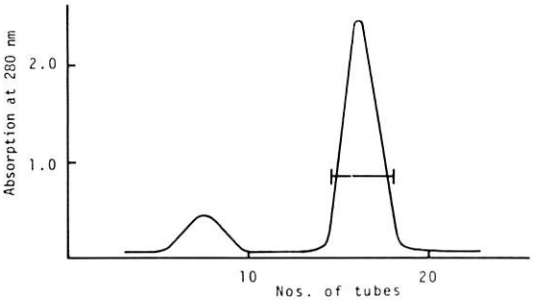

Fig. 1. Sepharose 4B elution pattern of a nephroblastoma LPfr *(left)*.

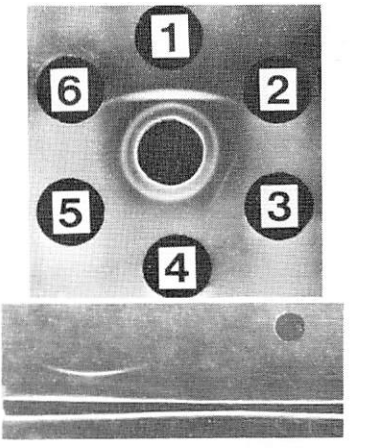

Fig. 2. Immunodiffusion pattern of anti-nephroblastoma LPfr serum reacted against LPfr of the tumor and normal organs *(right upper)*.

(1) nephroblastoma LPfr. (2) kidney LPfr. (3) liver LPfr. (4) spleen LPfr. (5) lung LPfr. (6) heart LPfr.

Fig. 3. Immunoelectrophoresis of a nephroblastoma LPfr *(right lower)*.

(39) Immunological detection of a tumor-specific antigen in the insolube fraction of human transitional cell carcinoma.

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In human transitional cell carcinoma (TCC), cellular immune responses directed against antigens associated with cancers of the urinary bladder in tumor-bearing individuals have been demonstrated by cytotoxicity assays. Some uncertainty as to the existence of such antigens was often raised. The identification of TSA in this tumor and its purification are as yet not established.

We prepared the LPfr preparation from 7 TCC of grade 1–3, 6 from the renal pelvis and 1 from the urinary bladder. Some of them were used to raise antisera in guinea pigs. The antisera were analyzed by immunodiffusion. The antiserum absorbed by spleen, liver, kidney, and urinary bladder mucosa produced a single precipitin band against all 7 TCC LPfr, but failed to react with normal adult organ LPfr and fetal organ homogenates tested. The seven precipitin bands thus formed showed identifying reactions among themselves. Tests with individual LPfr of various tumors showed that the reactive antigen was restricted to the TCC LPfr (Table 1). On immunoelectrophoresis the antigen migrated to the β_2-γ region. With immunofluorescence, the antiserum appeared to stain the cell membranes of the tumor cells (Fig. 1).

Table 1. Reactivity of LPfr from various tumors with absorbed anti-TCC LPfr serum by immunodiffusion

Tumor histologic type	No. positive/No. tested
TCC from renal pelvis	6 / 6
TCC from urinary bladder	1 / 1
Renal cell carcinoma	0 / 6
Nephroblastoma	0 / 1
Adenocarcinoma of stomach	0 / 6
Adenocarcinoma of stomach	0 / 3
Hepatoma	0 / 2
Squamous cell carcinoma of cervix	0 / 1
Squamous cell carcinoma of lung	0 / 1
Adenocarcinoma of lung	0 / 1
Small cell carcinoma of lung	0 / 1
Cystadenocarcinoma of ovary	0 / 2
Seminoma	0 / 1

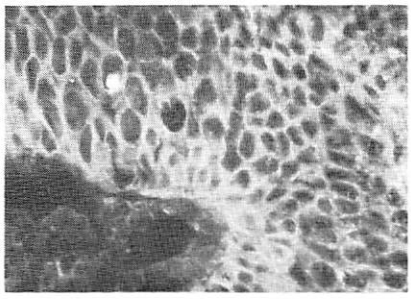

Fig. 1. Immunofluorescent staining of a TCC with absorbed antiserum.

(40) A tumor-specific antigen in the insolube fraction of human renal cell carcinoma.

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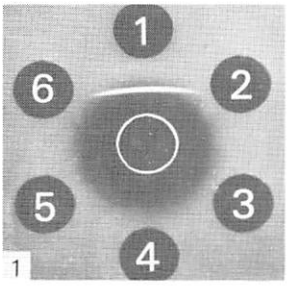
In human renal cell carcinoma (hypernephroma) cellular or humoral immune responses directed against the antigen(s) associated with the tumor by the tumor-bearing individuals have been repeatedly demonstrated. However, much still remain unclear about the tumor-associated or specific antigens. We have tried to look for a possible TSA in the insolube fraction of this tumor.

The LPfr preparations were prepared from 9 renal cell carcinomas and some of them were used to raise antisera in guinea pigs. The antisera obtained were analyzed by double immunodiffusion. After absorption by normal adult kidney, liver, serum, and fetal liver, the antisera reacted with 9 out of 9 renal cell carcinomas LPfr, showing reactions which enabled complete identification among all the bands which formed, but failed to react with LPfr of normal adult organs (Fig. 1), fetal organ homogenates (fig. 2), and LPfr of other tumors containing Wilms' tumor, transitional cell carcinoma, colonic adenocarcinoma, gastric adenocarcinoma, squamous cell carcinoma of the cervix, and hepatoma. On immunoelectrophoresis the antigen showed β_2-mobility. When tested by living cell membrane immunofluorescence, renal cell carcinoma cells exhibited a bright fluorescence outlining clearly the cell surface, suggesting its localization at the cell membrane (Fig. 3A), while normal tubular cells from the associated kidney remained unstained (Fig. 3B).



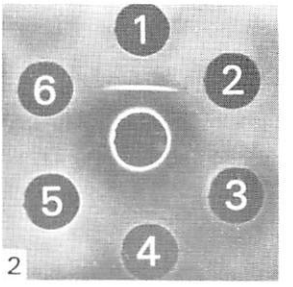
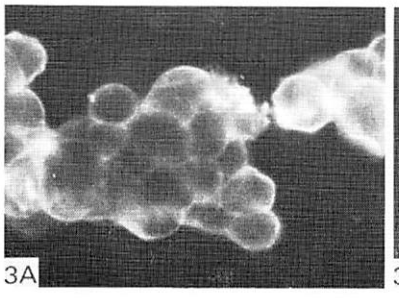
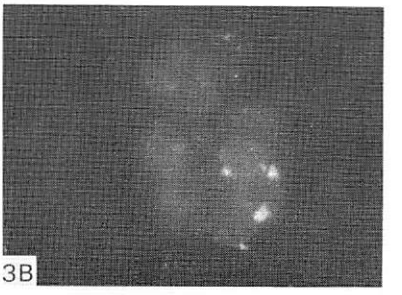


Fig. 1. Gel-diffusion patterns of absorbed antiserum against LPfr of renal cell carcinoma and normal adult organs.
(1) tumor. (2) kiney. (3) liver. (4) spleen. (5) lung. (6) heart.

Fig. 2. Gel-diffusion patterns of absorbed antiserum against renal cell carcinoma LPfr and fetal organ homogenates.
(1) tumor. (2) intestine. (3) liver. (4) spleen. (5) lung. (6) kidney.





Figs. 3A and B. Viable cell membrane immunofluorescence.
A. tumor cells showing the typical ring reaction.
B. negative renal tubular cells from the same donar.

(41) Identification of tumor-specific antigen in the insoluble fractions of human adenocarcinoma and squamous cell carcinoma of the lung.

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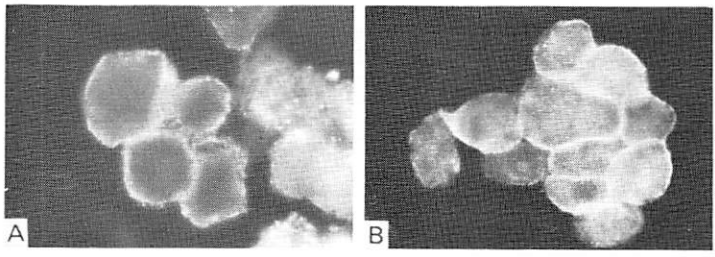
The existence of TSA which has the desired degree of specificity in human lung cancers except oat cell carcinoma is as yet not known. We have tried to look for a possible TSA in lung cancers. Antisera to LPfr from lung adenocarcinoma (ALPfr) and from lung squamous-cell carcinoma (SLPfr) were prepared in guinea pigs. Unabsorbed and variously absorbed antisera were analyzed by double immunodiffusion. After suitable absorption, the antisera each identified an antigen in LPfr of the corresponding carcinomas, but not in LPfr of normal organs and in fetal organ homogenates. Investigations of LPfr from various kinds of tumor showed these antigens were restricted to the corresponding tumors (Table 1). It thus seems that at least one adenocarcinoma-specific antigen (LASA) and one squamouscell carcinoma-specific antigen (LSSA) exist in lung cancers. On immunoelectrophoresis, LASA showed β_2-mobility, and LSSA β_1-mobility. When tested by viable cell membrane immunofluorescence, these antigens were found to be localized in the cell membranes of tumor cells (Fig. 1A and B). SDS-polyacrylamide-gel electrophoresis of dansylated antigenic fractions indicated an apparent molecular weight 6.1×10^4 D for both antigens.

Table 1. Reactivity of LPfr preparations from various tumors with anti-ALPfr and anti-SLPfr serum by immunodiffusion.

Tumor histologic type		No. positive / No. tested	
		anti-ALPfr	anti-SLPfr
Lung carcinoma	Adenocarcinoma	6 / 6	0 / 6
	Squamous cell carcinoma	0 / 7	7 / 7
	Large cell carcinoma	0 / 2	0 / 2
	Small cell carcinoma	0 / 3	0 / 3
Adenocarcinoma of stomach		0 / 3	0 / 3
Adenocarcinoma of colon		0 / 3	0 / 2
Adenocarcinoma of pancreas		0 / 1	0 / 1
Adenocarcinoma of prostate		0 / 1	0 / 1
Cystadenocarcinoma of ovary		0 / 1	0 / 1
Hepatoma		0 / 1	0 / 1
Renal cell carcinoma		0 / 4	0 / 5
Squamous cell carcinoma of cervix		0 / 1	0 / 1



Figs. 1A and B. Viable cell membrane immunofluorescence of tumor cells from adenocarcinoma (A) and from squamouscell carcinoma (B).

(42) Demonstration of a putative tumor-specific antigen in human pancreatic carcinoma by immunodiffusion.

K. Kitagawa, A. Matsuki and S. Okada

In human pancreatic carcinoma (PC) or in ascites fluid of PC the presence of oncofetal antigen(s) of glycoprotein nature has been identified. However, it is as yet not known whether the TSA exists in this carcinoma. We have tried to look for a possible TSA in PC by this method in our laboratory.

The LPfr of three PC were prepared and used to raise antiserum in guinea pigs. Unabsorbed antiserum produced precipitin bands with PC LPfr and normal organ LPfr (Fig. 1). The antiserum absorbed with normal liver, spleen, lung, and pancreas, produced a single precipitin line with PC LPfr but no reaction with normal adult organ LPfr tested (Fig. 2). The antiserum absorbed with CEA and fetal liver in addition to adult liver, spleen, lung, and pancreas reacted with LPfr from three PC but not with various fetal organ homogenates (Fig. 3). The three precipitin lines thus formed showed reactions of identity among themselves. The antigen showed immunoelectrophoretically $\beta 1$-mobility. Tests with LPfr of some tumors: colonic adenocarcinoma, gastric adenocarcinoma, lung adenocarcinoma, seminoma, and lymphosarcoma, with the absorbed antiserum were negative. It thus seems that at least one TSA exists in the insoluble fraction of PC but further examination will be required in this respect.

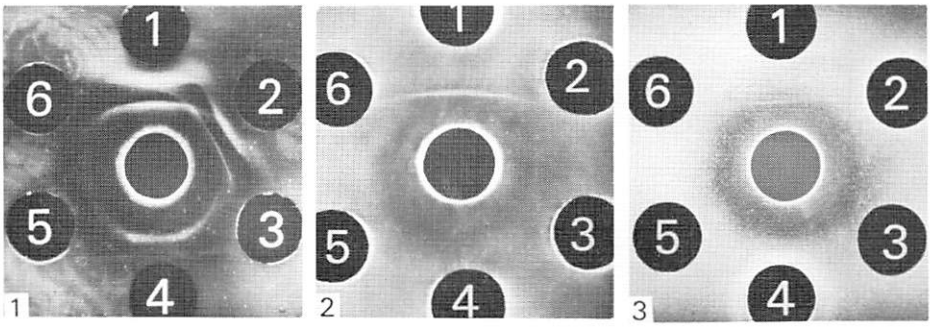


Fig. 1. Immunodiffusion: Comparative reaction of unabsorbed anti-PC LPfr serum to normal adult organ LPfr: 1) PC LPfr, 2) spleen LPfr; 3) kidney LPfr; 4) lung LPfr; 5) liver LPfr; 6) pancreas LPfr.

Fig. 2. Immunodiffusion: Comparative reaction of absorbed anti-PC LPfr serum to normal adult organ LPfr: 1) PC LPfr 2) spleen LPfr; 3) kidney LPfr; 4) lung LPfr; 5) liver LPfr; 6) pancreas LPfr.

Fig. 3. Immunodiffusion: Comparative reaction of absorbed anti-PC LPfr serum to fetal organ homogenates: 1) PC LPfr; 2) fetal spleen; 3) fetal kidney; 4) fetal lung; 5) fetal liver; 6) fetal pancreas.