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Immunology

Cell surface antigens of human renal cancer defined by mouse monoclonal antibodies: Identification of tissue-specific kidney glycoproteins

(serology/hybridoma/differentiation antigens)

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ABSTRACT **Seventeen monoclonal antibodies derived from fusions with spleen cells of mice immunized with established culture lines of renal cancers identified nine cell-surface antigenic systems. Six of the systems (gp160, S_{25}, gp120r, gp120nr, gp115, and V_1) represent antigens not previously described. The other three systems are related to HLA-A, -B, and -C heavy chain and A and B blood group antigens. The most restricted of the newly described antigens are gp160, S_{25}, and gp120r. These determinants are found only on cells of renal origin, both normal and malignant, and represent differentiation antigens of human kidney. In addition to the difference in the molecular weight of two of these antigens, gp160, S_{25}, and gp120r can be distinguished on the basis of differential expression on a panel of cultured renal cancers and normal kidney epithelium and fetal kidney cells. Glycoproteins bearing gp120r share a determinant with renal gp120nr (as indicated by sequential precipitations with monoclonal antibodies that detect gp120r and gp120nr), but gp120nr is found on a broader range of cell types, including fibroblasts and cell lines derived from lung, bladder, and colon cancers. The two other new systems, gp115 and V_1, have characteristics of broadly occurring differentiation antigens but can be distinguished from each other and from gp120nr by differences in molecular weight, heat stability (V_1 is a heat-stable determinant), and differential expression on cell types of diverse origin.**

We recently described our initial analysis of cell surface antigens of human malignant melanoma identified by mouse monoclonal antibodies (Abs) (1). This report summarizes the results of a comparable analysis of human renal cancer.

MATERIALS AND METHODS

Tissue Culture. The renal cancer cell lines (2) and tumor cell lines (3) have been described. Methods for the short-term culture of normal kidney epithelium have also been described (2). Cultures were maintained in Eagle's minimal essential medium supplemented with 2 mM glutamine, 1% nonessential amino acids, 100 units of penicillin per ml, 1 μg of streptomycin per ml, and 10% (vol/vol) fetal bovine serum. Cultures were regularly tested for mycoplasma, fungi, and bacteria, and contaminated cultures were discarded.

Serological Procedures. The mouse mixed hemadsorption assay (M-MHA) was performed by the method of Fagraeus *et al.* (4), as modified to detect mouse antibody. Serological procedures for direct test and absorption analysis are described in refs. 1, 2, and 3.

Immunizations. (BALB/c × C57BL/6)F_1 female mice were immunized with established renal cancer cell lines (see Table 1). For the initial immunization, 1×10^7 renal cancer cells were injected subcutaneously without adjuvant. Subsequent immunizations were carried out at intervals of 3–4 weeks by intraperitoneal inoculation of 1×10^7 renal cancer cells. Immunized mice were sacrificed 3 days after the last immunization.

Derivation of Mouse Abs. The fusion of immune spleen cells with mouse myeloma MOPC-21 NS/1 cells was performed as described (1, 5). Fused cells ($5–8 \times 10^5$) in 1 ml of selective medium containing hypoxanthine, aminopterin, and thymidine were added to wells of tissue culture plates (Costar no. 3524, 24 wells per plate). Hybridoma cultures were subcloned at least three times by limiting dilution on a feeder layer of $1–3 \times 10^5$ mouse peritoneal macrophages per ml. Culture supernatants were monitored for antibody activity on a panel of cultured cells consisting of two renal cancer cell lines (including the immunizing line), AJ astrocytoma, SK-MEL-33 and -37 melanomas, ME-180 cervix cancer, WI-38 fetal cells, VERO, adult and fetal kidney epithelium, and fetal brain cells. Antibody subclass was determined by double diffusion in agar with anti-Ig heavy chain specific reagents (Bionetics, Kensington, MD). Cultures of cloned hybridomas were injected subcutaneously into *nu/nu* mice (Swiss background) and were also stored in liquid nitrogen. Sera from mice with progressively growing tumors were collected, stored at −70°C, and used for serological and biochemical characterization.

Immunoprecipitation Procedures. Cells were metabolically labeled with [^{3}H]glucosamine in complete Eagle's medium containing 15 μCi of [^{3}H]glucosamine (New England Nuclear; 30–60 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) per ml for 48 hr at 37°C; the labeled cells were extracted with 0.5% Nonidet P-40 (NP-40) in Tris buffer as described (6) except that the 3 M KCl treatment was omitted. Immunoprecipitation was carried out by mixing a portion of the cell extract (1×10^5 cpm) with 2 μl of mouse serum and 20 μl of rabbit anti-mouse Ig serum (Cappel Laboratories, Cochranville, PA) in Tris buffer. Immune complexes were isolated by using *Staphylococcus aureus* and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis as described (1, 7). [^{35}S]Methionine-labeled samples were immunoprecipitated in a similar manner, except that Sepharose-rabbit F(ab')$_2$ anti-mouse IgG was used for isolating the complexes. To determine the pI of the antigens, immunoprecipitates were examined by two-dimensional electrophoresis by the O'Farrell procedure (8), modified as described (6).

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Abbreviations: Ab, monoclonal antibody; M-MHA, mouse mixed hemadsorption assay; NP-40, Nonidet P-40; EBV, Epstein–Barr virus.

Table 1. Derivation of mouse hybridomas producing Abs reacting with surface antigens of human renal cancer cells

Exp.	Renal cancer cell line used for immunizations	Immunizations, no.	Results of initial fusion and antibody screening: Growth/wells, no./no.	Results of initial fusion and antibody screening: Positive wells, no.	Clones isolated and analyzed, no.	Abs characterized
1	SK-RC-7	8	462/480	450	6	$S_1(\gamma1)$, $S_4(\gamma2a)$*, $S_6(\gamma1)$*, $S_7(\gamma2a)$, $S_8(\mu)$*, $S_{11}(\gamma2a)$
2	SK-RC-7	2	175/336	50	7	$S_{21}(\gamma1)$*, $S_{22}(\gamma1)$*, $S_{23}(\gamma1)$*, $S_{24}(\gamma1)$, $S_{25}(\gamma1)$*, $S_{26}(\gamma1)$, $S_{27}(\gamma1)$
3	SK-RC-7	1	43/320	0	0	None
4	SK-RC-6	8	64/72	64	2	$V_1(\gamma1)$*, $V_2(\gamma1)$
5	SK-RC-28	3	225/476	212	2	$M_1(\gamma1)$, $M_2(\mu)$*

* Prototype Abs (see Tables 2 and 3).

RESULTS

From the five fusions of NS-1 myeloma with spleen cells from mice immunized with three different renal cancer cell lines, 17 antibody-producing clones were selected for detailed analysis (Table 1). The serological specificity of these antibodies was tested on a panel of 47 established cell lines [13 renal cancers, 6 melanomas, 5 astrocytomas or neuroblastomas, 15 epithelial cancers, 5 B-cell lines, 2 T-cell lines (MOLT-4 and T-45), and monkey kidney cells (VERO)]. In addition, the antibodies were tested against short-term cultures of normal kidney epithelium, fibroblasts, and fetal tissues (brain, fibroblasts, and kidney). Human, sheep, rat, and bovine erythrocytes were also examined. In most cases, serological analysis consisted of both direct and absorption tests.

These serological studies in conjunction with immunochemical analysis defined nine distinct antigenic systems. Three systems (gp160, S_{25}, and gp120r) were restricted to normal and malignant renal cells, three systems (gp120nr, gp115, and V_1) were more widely distributed, and three systems were identified as HLA-A, -B, -C heavy chain and A and B blood group antigens.

gp160 Antigenic System. Five Abs in this series (S_4, S_7, S_{11}, S_{24}, and M_1) identified a 160,000-dalton glycoprotein that showed a high degree of specificity for human kidney cells. Immunoprecipitation tests with Ab S_4, the prototype antibody detecting gp160, are illustrated in Fig. 1. gp160 is a rather basic component with pI > 7.5. By M-MHA tests, gp160 could be demonstrated on all cultures of normal kidney epithelium, 2 of 3 cultures of fetal kidney, and 7 of 13 established lines of renal cancer (Table 2). These results were confirmed in absorption tests. No other cell type, normal or malignant, was found to express the gp160 antigen, including VERO, a cell line derived from monkey kidney.

S_{25} Antigenic System. The antigen detected by Ab S_{25} also is restricted to human cells of renal origin (Table 2). The S_{25} determinant is heat labile, suggesting that it resides on a protein or glycoprotein, but Ab S_{25} did not precipitate any detectable component from [^{35}S]methionine-labeled or [^{3}H]glucosamine-labeled SK-RC-7 cells. Comparison of the S_{25} and the gp160 phenotypes of different renal cancer lines and cultures of normal kidney clearly distinguished these two systems. For example, SK-RC-6 and A-498 are $gp160^+/S_{25}^-$ and SK-RC-8 is $gp160^-/S_{25}^+$. In addition, all nine cultures of normal kidney epithelium were $gp160^+$, whereas five of these cultures lacked S_{25} expression.

gp120r and gp120nr Antigenic Systems. Five Abs (S_{23}, S_{26}, S_{27}, S_6, and S_1) immunoprecipitated a 120,000-dalton glycoprotein from [^{35}S]methionine- or [^{3}H]glucosamine-labeled lysates of SK-RC-7 cells (Fig. 1). Analysis under reducing and nonreducing conditions gave the same results. The pIs of gp120 identified by prototype Ab S_6 and Ab S_{23} were identical (4.9–5.2). A further indication of the relatedness of the gp120 components identified by these two groups of Abs came from sequential immunoprecipitation tests. Pretreatment of [^{3}H]glucosamine-labeled lysates of SK-RC-7 with Ab S_6 removed all antigen reactive with Ab S_{23}. In contrast, M-MHA tests and absorption analysis (Table 2) showed that these gp120 antibodies identified two serologically distinct gp120 epitopes that distinguish two classes of gp120 molecules: gp120r (restricted) and gp120nr (nonrestricted).

gp120r, identified by Ab S_{23}, had a highly restricted distribution, expression being limited to normal kidney epithelium and certain renal cancers. The other gp120 epitope, gp120nr, identified by Ab S_6, was found on a wide range of cultured cells, including fetal and adult fibroblasts and cell lines derived from lung, bladder, and colon cancers. gp120r and gp120nr determinants differ in their expression on renal cancer cell lines: all cell lines carry the gp120nr epitope, whereas SK-RC-2, -21, -29, and CaKi-1 lack gp120r determinants. The specificity of Ab S_{23} for cells of renal origin resembles the reactivity of Ab S_{25} and, most particularly, antibodies identifying the gp160 system. However, in addition to the molecular weight differences in the gp160 and gp120 antigens, these three kidney-specific antigenic systems can be distinguished on the basis of absorption analysis with selected normal or malignant kidney cells—e.g., SK-RC-

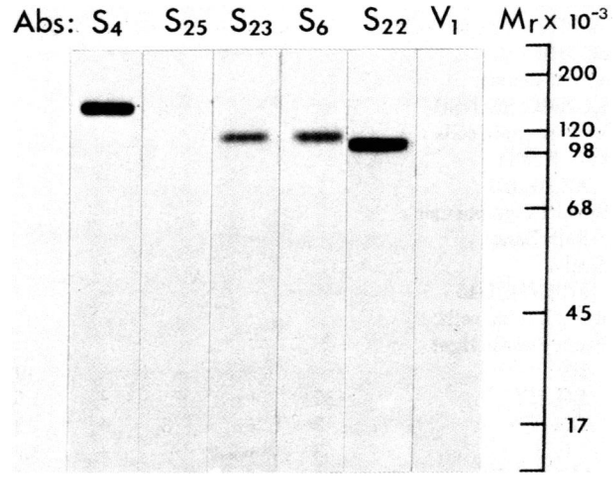

FIG. 1. Autoradiogram of [^{3}H]glucosamine-labeled glycoproteins from lysates of SK-RC-7 immunoprecipitated by mouse monoclonal antibodies to renal cancer cells and analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis (reduced samples). Molecular weight markers: β-galactosidase (120,000), phosphorylase (98,000), bovine serum albumin (68,000), ovalbumin (45,000), and myoglobin (17,000).

Table 2. Serological characterization of seven prototype mouse Abs detecting surface antigens on human renal cancer cells

Cells	Ab S_4		Ab S_{25}		Ab S_{23}		Ab S_6		Ab S_{22}		Ab V_1		Ab S_{21}	
	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.
Epithelial cancers:														
Renal														
SK-RC-1 (AA)	50	+	−	+	1	+	50	+	−	+	500	+	50000	+
SK-RC-2 (AB)	−	−	−	−	−	−	50	+	−	+	500	+	5000	+
SK-RC-4 (AE)	50	+	500	+	50	+	50	+	5000	+	10000	+	5000	+
SK-RC-6 (AG)	50	+	−	−	10		1000	+	5	+	10000	+	100000	+
SK-RC-7 (AX)	50	+	500	+	1	+	500	+	10000	+	10000	+	50000	+
SK-RC-8 (BE)	−	−	1	+	1	+	50	+	50	+	50	+	500	+
SK-RC-9 (BM)	−	−	−	+	−	+	500	+	50	+	5000	+	50000	+
SK-RC-11 (BZ)	5	+	−	+	1	+	1000	+	−	+	10000	+	50000	+
SK-RC-21 (EB)	−	−	−	−	−	−	500	+	−	+	1	+	50000	+
SK-RC-28 (EU)	50	+	−	+	500	+	5000	+	−	+	500	+	100000	+
SK-RC-29 (BW)	−	−	−	−	−	−	50	+	−	+	5000	+	50000	+
A-498	10	+	−	−	−	+	50	+	−	+	10000	+	100	+
CaKi-1	−	−	−	−	−	−	50	+	−	+	10000	+	100	+
Bladder														
RT-4	−	−	−	−	−	−	−	−	−	+	5000	+	50	+
5637	−	−	−	−	−	−	−	−	−	+	−	−	10	+
T-24	−	−	−	−	−	−	5	+	−	+	−	−	10000	+
253J	−	−	−	−	−	−	5	+	−	+	5000	+	5000	+
Breast														
AlAb	−	−	−	−	−	−	−	−	−	−	5	+	500	+
BT-20	−	−	−	−	−	−	−	−	−	−	−	−	50	+
MCF-7	−	−	−	−	−	−	−	−	−	−	10000	+	1000	+
SK-BR-3	−	−	−	−	−	−	−	−	−	−	10000	+	10	+
Cervix														
ME-180	−	−	−	−	−	−	−	−	−	−	−	+	−	+
Colon														
HT-29	−	−	−	−	−	−	5	+	−	−	−	+	50	+
SW-1222	−	−	−	−	−	−	−	−	−	−	500	+	5	+
Lung														
SK-LC-LL	−	−	−	−	−	−	−	−	−	+	1	+	5	+
SK-LC-6	−	−	−	−	−		50	+	−	−	10000	+	50000	+
Ovary														
SK-OV-3	−	−	−	−	−	−	−	−	0.5	+	−	+	50	+
Testicular														
SK-GR-1	−	−	−	−	−	−	−	−	−	−	−	+	1	+
Astrocytomas:														
AJ, AS, BE	−	−	−	−	−	−	5	+	−	+	−	−	500	+
Melanomas:														
SK-MEL-13,28,29,37,41	−	−	−	−	−	−	−	−	−	−	5000	+	5000	+
SK-MEL-19	−	−	−	−	−	−	−	−	−	−	5000	+	−	+
Neuroblastomas:														
SK-NMC, SK-NSH	−	−	−	−	−	−	−	−	−	−	1	+	100	+
Lymphoblastoid cells:														
EBV B cells														
AX,BE,EU		−		−		−		−		−		+		+
Burkitt's lymphomas														
Raji, Daudi		−		−		−		−		−		+		+
T cells														
MOLT-4, T-45		−		−		−		−		−		−		+
Normal human cells:														
Kidney epithelium														
ID			−	−	10	+	5	+	1	+	5	+	25	+
EQ, HY	10	+	−	−	1.5	+	5	+	1	+	5	+	25	+
GM, FR	3	+	3	+	3	+	5	+	1	+	5	+	25	+
EI, LJ	3	+	−	+	1.5	+	5	+	1	+	5	+	25	+
EG, GR, IB	0.5	+	−	−	0.5	+	5	+	1	+	5	+	25	+
Fetal kidney														
C-4, C-8	0.5	+	2	+	−	−	>5	+	>5	+	>5	+	>5	+
C-6	−	−	2	+	−	−	>5	+	>5	+	>5	+	>5	+

Table 2. (*Continued*)

Cells	Ab S_4		Ab S_{25}		Ab S_{23}		Ab S_6		Ab S_{22}		Ab V_1		Ab S_{21}	
	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.
Adult skin fibroblasts	–	–	–	–	–	–	5	+	–	–	–	–	5	+
Fetal lung fibroblasts	–	–	–	–	–	–	1.5	+	0.5	+	0.5	+	10	+
Fetal brain	–	–	–	–	–	–		–		+		–	10	+
Erythrocytes		–		–		–		–		–		–		–
Xenogeneic cells:														
Monkey kidney VERO	–	–	–	–	–	–	5	+	–	–	–	–	–	–
Sheep erythrocytes		–		–		–		–		–		–		–

Under "Titer," – indicates no reaction in direct tests at a dilution of 1:200. Abs., absorption tests. Sera (diluted to end point) were absorbed with the indicated cell type and tested for residual activity for SK-RC-7 (Ab S_4, Ab S_6, Ab S_{22}, Ab S_{21}), SK-RC-4 (Ab S_{25}), SK-RC-6 (Ab V_1), or SK-RC-28 (Ab S_{23}) target cells; +, complete absorption; –, no absorption.

6 and A-498 are $gp160^+/S_{25}^-/gp120r^+$; fetal kidney is $gp160^+$ or $gp160^-/S_{25}^+/gp120r^-$.

gp115 Antigenic System. Ab S_{22} immunoprecipitated a 115,000-dalton glycoprotein from [^{3}H]glucosamine- or [^{35}S]methionine-labeled lysates of SK-RC-7 cells under both reduced and nonreduced conditions (Fig. 1). In direct M-MHA tests, high reactivity (titers, 1×10^{-3}–1×10^{-7}) was restricted to certain renal cancer cells and normal kidney epithelium (Table 2). Absorption analysis, however, revealed that the gp115 antigen was expressed by various other cell types.

V_1 Antigenic System. Ab V_1 did not immunoprecipitate any labeled component from [^{3}H]glucosamine- or [^{35}S]methionine-labeled lysates of SK-RC-7 cells. Absorption tests indicated that the antigen is heat stable (5 min at 100°C), suggesting that it may be a glycolipid. Two features of the V_1 (Table 2) system are of particular interest: (*a*) it identifies a subset of bladder and breast cancers that do not express V_1, and (*b*) V_1 is not found on astrocytomas, whereas melanomas are strong V_1 expressors. This clear distinction between astrocytoma and melanoma, whose embryonic derivations are closely related, has not been seen with other Abs.

HLA Heavy Chain. Ab S_{21} immunoprecipitated a 45,000- and a 12,000-dalton component from [^{35}S]methionine-labeled SK-RC-7 lysates. The determinant detected by Ab S_{21} in direct and absorption tests was present on virtually every human cell type with the exception of human erythrocytes (Table 2). Of all the human cultured cells tested, the only cell lines not reactive with Ab S_{21} in direct M-MHA tests were ME-180 and SK-MEL-19; the SK-MEL-19 melanoma cell line is known from previous work to express little or no HLA-A, -B, -C antigens. The molecular weights of the components precipitated by Ab S_{21} and the results of the serological survey of human cells indicated that Ab S_{21} detected HLA but did not distinguish between a determinant on the heavy chain or on the β_2m chain. The fact that isolated human β_2m did not inhibit the reactivity of Ab S_{21} suggests specificity for HLA heavy chain.

A and B Blood Group Antigens. Two of the three renal cancer lines used for immunization (Table 1) express blood group A or B antigens on their cell surfaces; SK-RC-7 is B^+ and SK-RC-28 is A^+. SK-RC-6 is derived from a type O individual and is negative for A and B reactivities. To detect Abs reacting with blood group antigens, hybridoma supernatants were screened for hemagglutinating antibody by using A, B, AB, or O erythrocytes. B (but not A) agglutinating activity was found in 4 of 462 supernatants from the anti-SK-RC-7 fusion, and A (but not B) agglutinating activity was found in 3 of 225 supernatants from the anti-SK-RC-28 fusion. No agglutination of type O erythrocytes was found in supernatants from anti-SK-RC-7, -28 or -6 fusions. Two monoclonal antibodies with agglutinating activity were derived from these fusions. The hemagglutination titer of Ab M_2 (*nu/nu* serum) for A and AB erythrocytes was 10^{-4}; B erythrocytes were not agglutinated by Ab M_2. The hemagglutination titer of Ab S_8 (*nu/nu* serum) for B and AB erythrocytes was 4×10^{-5}; A type erythrocytes were not agglutinated by Ab S_8. Table 3 summarizes inhibition tests with Ab S_8 and Ab M_2 using glycoprotein and mucin extracts having A, B, H, and Lewis^a blood group reactivity. The results confirmed the A specificity of Ab M_2 and the B specificity of Ab S_8.

DISCUSSION

This study of renal cancer and our recent study of melanoma (1) have generated a series of mouse Abs that define 12 new systems of human cell surface antigens. Six of these have been identified as glycoproteins (gp95, gp150, gp160, gp120r, gp120nr, and gp115), three are heat-labile antigens that could not be immunoprecipitated from labeled cell extracts (S_{25}, M_{19}, and R_8), and three are heat-stable antigens, presumably glycolipids (O_5, R_{24}, and V_1). The use of a standard panel of cultured human cells allows ready comparisons of the reactivity of these monoclonal antibodies in direct serological tests and absorption analysis, and each of the antigenic systems has a distinct pattern of distribution on the cell panel, in terms of both qualitative and quantitative expression of antigens. On the basis of their distribution on different cell types, these 12 antigenic systems can be further classified into three groups: (*i*) those with characteristics of restricted differentiation antigens (e.g., the renal-specific gp160, S_{25}, and gp120r antigens and the R_{24} antigen of

Table 3. Ab M_2 and Ab S_8: Inhibition tests with blood group glycoproteins*

Glycoprotein	Amount of glycoprotein required for inhibition, μg/ml: Ab M_2†	Ab S_8‡
Human A glycoprotein (Sullivan)	1.3	120.0
Human B glycoprotein (Beach)	450.0	1.2
Human O(H) glycoprotein (Tighe)	246.0	123.0
Human Le^a glycoprotein (N-1)	475.0	237.0
Porcine A + H gastric mucin	3.0	550.0
Porcine A gastric mucin (67)	4.6	437.0
Porcine H gastric mucin (66)	437.0	449.0

* Blood group glycoproteins were kindly provided by E. A. Kabat and have been described (9).

† Agglutination assay with A erythrocytes using 1:1000 dilution of Ab M_2.

‡ Agglutination assay with B erythrocytes using 1:10,000 dilution of Ab S_8.

melanoma and melanocytes), (*ii*) more broadly represented differentiation antigens (e.g., gp95, gp150, M_{19}, gp120nr, and V_1), and (*iii*) antigens expressed by every human cell type tested (e.g., O_5 species antigen).

The next step in the analysis of these antigens will be to assess their cellular distribution in fixed or frozen tissue sections by immunofluorescence or immunoperoxidase methods. Cell lines derived from different individuals with the same tumor type can be distinguished on the basis of typing for these various differentiation antigens, and it will be important to see whether this is also true for tumor specimens prior to culture. For instance, will it be possible to identify a subset of renal cancers on the basis of gp160 typing (corresponding to the $gp160^-$ renal cancer cell lines)? If it is possible, what relation will the gp160 phenotype have to other biological features of the tumor? A clue in this regard comes from the finding that the cell lines derived from stage I/II renal cancer (confined to the kidney) were $gp160^+$, whereas cell lines from metastatic renal cancers were $gp160^-$. Whether this indicates that cancer cells developing metastatic potential lose gp160 expression, or that $gp160^+$ and $gp160^-$ renal cancers are derived from separate cell lineages, needs to be determined. Identifying the cell types in normal kidney that express gp160 and other antigens found on renal cancer should also give information about the cellular origins of renal cancer. These serological probes that identify kidney-specific antigens should be of particular interest in the study of kidney structure and function. In addition, some of the more broadly reacting antibodies will be useful in studying other tumors—e.g., V_1 which distinguishes astrocytomas from melanomas.

The importance of parallel biochemical and serological characterizations of antigens identified by Abs is illustrated by the analysis of gp120r and gp120nr. Five Abs in this series immunoprecipitated a 120,000-dalton component from labeled extracts of SK-RC-7 renal cancer cells. Preclearing the extract with one of these Abs (Ab S_6) removed the 120,000-dalton component identified by Ab S_{23}, indicating that the two Abs were reacting with the same molecule. However, the antigenic determinant detected by Ab S_6 and Ab S_{23} can be distinguished in M-MHA tests and absorption analysis. Ab S_{23} detected a kidney-specific antigen, whereas Ab S_6 reacted with a much broader range of cell types. These results can be explained by postulating two species of gp120 molecules, both carrying the epitope identified by Ab S_6 but only one with the epitope identified by Ab S_{23}. In agreement with this interpretation, supernatants after clearing with Ab S_{23} still reacted with Ab S_6, even though no antigen precipitating with Ab S_{23} remained. The epitope identified by Ab S_{23} is found only on cells of renal origin and, because of this restricted distribution, it is referred to as gp120r. The more widely distributed epitope has been designated "nr" to indicate its nonrestricted nature. gp120r and gp120nr may be the products of two separate genes or of a single gene whose product is modified in renal cells. Similar, although less striking, discrepancies in the cellular distribution of antigens identified by different monoclonal antibodies immunoprecipitating gp95 or gp150 molecules have also been explained on the basis of different epitopes being recognized (1).

None of the mouse Abs identified in this study of renal cancer or in our study of melanoma recognized antigens that are tumor-specific. Although claims have been made for the tumor specificity of mouse Abs reacting with human tumor cells, none of these studies stands up to serological scrutiny. Nevertheless, the hybridoma technology has had too limited an application in the analysis of human cancer to permit any conclusions about the existence of tumor-specific antigens. With regard to human tumor antigens recognized by mouse Abs, the continuing problem will be to distinguish between tumor-specific reactions and reactions due to highly restricted differentiation antigens, especially because it is so difficult to identify for study the appropriate normal cell counterpart for most malignant cells. The same problem will have to be dealt with when human monoclonal antibodies become available for the study of human cancer.

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