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# Quantitative analysis of melanoma-associated antigen p97 in normal and neoplastic tissues

(human tumor antigen/monoclonal antibody/hybridoma/serology/radioimmunoassay)

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ABSTRACT **We have used two highly sensitive assays to quantitate p97, a protein associated with human melanoma, in cultured cells and normal adult, fetal, and neoplastic tissues. To measure p97 at the surface of intact cells, radiolabeled Fab fragments of a monoclonal antibody specific for p97 were used in a binding assay. To measure p97 in detergent-solubilized membrane preparations, we used a novel double-determinant immunoassay that uses two monoclonal antibodies to two distinct antigenic determinants of p97. These assays revealed that although p97 is present in small amounts in normal adult tissues, it is present in much larger amounts in most melanomas, in some other tumors (both benign and malignant), and in certain fetal tissues. We conclude that monoclonal antibodies to p97 may prove to be of value for the diagnosis and therapy of melanoma.**

Work in our laboratory has led to the identification of p97, a 97-kilodalton cell-surface protein of human melanoma (1, 2). Radiolabeled monoclonal antibody to p97 was used to test normal and neoplastic cells in binding assays (1), which showed p97 to be present on most melanomas, in smaller amounts on some carcinomas, but undetectable on fibroblasts or B-cell lines. Immunoprecipitation of radiolabeled membrane lysates demonstrated the presence of p97 in biopsy samples of several melanomas and a breast carcinoma but not in eight normal adult tissues.

Immunoprecipitation tests of a larger number of normal and neoplastic tissues were consistent with these findings. p97 was not detected in normal adult tissues, but it was found in most melanomas, in some carcinomas, in a benign nevus, and in certain fetal tissues (unpublished data).

In this study we describe a binding assay with radiolabeled Fab fragments of monoclonal anti-p97 antibody, which we used to measure p97 at the surface of intact cells, and a new, highly specific double-determinant immunoassay (DDIA), which was used to assay p97 in tissue extracts. We found, in fact, that normal adult tissues do express small amounts of p97; however, many melanomas and certain fetal tissues express much larger amounts of p97.

## MATERIALS AND METHODS

**Cells.** Human and mouse cells were cultured as described (3). The origins of the various normal and neoplastic cells tested are indicated in the footnotes to Tables 1 and 2.

**Tissues.** Normal and neoplastic adult tissues were obtained either at the time of surgery or autopsy. Fetal tissues were obtained from aborted fetuses. Tissue specimens were kept on ice for less than 4 hr before they were frozen and stored in liquid nitrogen.

**Monoclonal Antibodies.** From ascites fluid of BALB/c mice that had been inoculated intraperitoneally with hybridoma 96.5(2), antibody 96.5 was purified by affinity chromatography on *Staphylococcus aureus* protein A coupled to Sepharose CL-4B (Pharmacia) (4). $IgG_2$ antibody (22 mg) was obtained from 4 ml of ascites fluid (protein concentrations were determined spectrophotometrically, based on an $A_{280}^{1\%}$ of 14.0). Antibody 4.1 (1) was purified similarly, as was $IgG_2$ from normal BALB/c serum and antibody 96.6 from spent culture medium from hybridoma 96.6 (2). Antibody 118.1 was used in the form of spent culture medium from hybridoma 118.1 (about 5 μg of antibody per ml). Antibody W6/32, specific for HLA heavy chain, was obtained from Accurate Scientific and Chemical (Hicksville, NY).

**Fab Fragments.** Antibody 96.5 (15 mg) was incubated with papain (150 μg) in 4 ml of phosphate-buffered saline (pH 7.2) containing 10 mM cysteine and 2 mM EDTA under nitrogen at 37°C for 3 hr. Iodoacetamide was added to a final concentration of 50 mM, and the digest was gel-filtered on a 20-ml column of Sephadex G-25 (superfine; Pharmacia) equilibrated with saline. Fc fragments were removed by passage through a 5-ml column of protein A coupled to Sepharose CL-4B. The yield of Fab 96.5 was 10 mg.

**Radioiodination.** Proteins were radioiodinated by the method of McConahey and Dixon (5). Protein (36 μg) was incubated with 1 mCi (1 Ci = $3.7 \times 10^{10}$ becquerels) of Na $^{125}$I or Na $^{131}$I (Amersham) and 10 μg of chloramine T in 400 μl of phosphate-buffered saline for 10 min at 0°C. The reaction was stopped by adding 10 μg of sodium metabisulfite, and the labeled protein was gel-filtered on a 5-ml column of Sephadex G-25 (superfine), pretreated with 100 μl of fetal calf serum, and then equilibrated with saline. The specific radioactivity of the labeled protein was calculated from the amount of protein labeled, the amount of radioactivity incorporated, and the yield of protein from the Sephadex column (shown previously to be greater than 90%). The specific radioactivity was approximately 40,000 cpm/ng for $^{125}$I and 10,000 cpm/ng for $^{131}$I. Labeled proteins were stored at −70°C in saline containing 10% (vol/vol) fetal calf serum.

**Binding Assays.** Adherent cultured cells were trypsinized before use to give a suspension of viable cells. The cells were incubated with $10^6$ cpm of $^{125}$I-labeled Fab 96.5 and $2.5 \times 10^5$ cpm of $^{131}$I-labeled Fab fragments of normal $IgG_2$ (about 25 ng of each) in 100 μl of heat-inactivated fetal calf serum in a 15-ml centrifuge tube at 0°C, were washed three times with 10 ml of phosphate-buffered saline containing 1% heat-inactivated fetal calf serum, and were counted in a Beckman Auto-Gamma scintillation counter. The number of cells tested ranged from $2 \times 10^4$ for melanoma cells to $2 \times 10^6$ for lymphoid cells.

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Abbreviation: DDIA, double-determinant immunoassay.

**Membrane Lysates.** Adherent cultured cells (approximately $10^7$) were washed with saline and incubated for 5 min at 0°C with 10 ml of 1 mM $NaHCO_3$ buffer containing 1 mM phenylmethylsulfonyl fluoride. The hypotonically swollen cells were scraped from the culture flask and disrupted with five strokes of the B pestle of a Dounce homogenizer. The nuclei were removed by centrifugation for 2 min at 2000 × *g*. The microsome fraction was pelleted by centrifugation for 10 min at 50,000 × *g*, suspended in 0.5 ml of buffer (20 mM Tris·HCl/100 mM NaCl/1 mM EDTA/0.5% Nonidet P-40, pH 8.0), and spun for 10 min at 300,000 × *g*. The supernatant, which contained solubilized membrane proteins, was stored at −70°C.

Tissue samples (50 mg) were minced with scissors and disrupted with the B pestle of a Dounce homogenizer in 5 ml of 1 mM $NaHCO_3$ containing 1 mM phenylmethylsulfonyl fluoride at 0°C. The microsome fraction was purified and lysed as described above. The protein concentrations of the lysates (0.1–1 mg/ml) were determined as described (6).

**DDIA.** Membrane lysates (20–100 μl) were incubated with 10 μl of spent culture medium from hybridoma 118.1 (about 50 ng of antibody) in V-bottom microtest wells (Dynatech Laboratories, Alexandria, VA) at 20°C for 15 min. Formalin-fixed *S. aureus* (obtained as a frozen cell suspension from New England Enzyme Center, Boston, and diluted with the Tris/NaCl/EDTA/Nonidet P-40 buffer to 25 mg/ml) was added (20 μl), and the incubation was continued for 15 min. The bacteria were pelleted by centrifuging the plate for 2 min at 1500 × *g* and the supernatants were aspirated. The bacteria were resuspended in 40 μl of the buffer containing 0.1% $NaDodSO_4$ and $10^6$ cpm (25 ng) of $^{125}$I-labeled Fab 96.5. After 15 min at 0°C, the bacteria were washed three times with 100 μl of the Tris/NaCl/EDTA/Nonidet P-40 buffer and were transferred to tubes for $^{125}$I determination.

IgG in the test samples interferes with the assay by competing with antibody 118.1 for binding to the *S. aureus*. Consequently, tissue lysates were tested for competition as described (2), except that the same amount of *S. aureus* as in the DDIA was used; in most cases the tissue lysates were found to be negative. Human serum, which contains a high concentration of IgG, cannot be tested directly. IgG was removed from serum samples before testing by passing 0.5 ml through a 1-ml column of *S. aureus* protein A coupled to Sepharose CL-4B equilibrated with the Tris/NaCl/EDTA/Nonidet P-40 buffer.

## RESULTS

**Monoclonal Antibodies to p97.** p97 originally was identified by a monoclonal $IgG_1$ antibody produced by a hybridoma, 4.1 (1). Shortly afterward we identified a second hybridoma, 96.5, which produces an $IgG_2$ antibody (2). Sequential immunoprecipitation experiments (7) showed that antibody 96.5 also binds p97. Antibody 96.5 competes with antibody 4.1 for binding to p97, so the two antibodies may recognize the same antigenic determinant. The determinant recognized by antibody 96.5 has been designated $p97^a$. Recently we identified a third hybridoma, 118.1 (unpublished data). Sequential immunoprecipitation showed that antibody 118.1 binds p97. However, antibody 96.5 does not compete with antibody 118.1. Thus, antibody 118.1 identifies a second determinant of p97, designated $p97^b$.

**Binding Studies of Intact Cells with $^{125}$I-Labeled Fab 96.5.** Expression of p97 at the surface of cultured cells and lymphocytes was examined by using $^{125}$-labeled Fab 96.5 in a binding assay. Table 1 shows the results expressed as ng of Fab bound per $10^7$ cells. By testing graded numbers of cells, we established that the amount of bound $^{125}$I-labeled Fab 96.5 was proportional to the number of cells tested up to about 40,000 cpm (about 1 ng). The amount of Fab 96.5 bound per cell was thus calculated from values within that range ($2 \times 10^4$ melanoma cells, $2 \times 10^5$ fibroblasts, and 1 or $2 \times 10^6$ lymphoid cells). The amount of Fab 96.5 bound ranged from 490 ng per $10^7$ cells for melanoma 1477 to 0.06 ng per $10^7$ cells for peripheral blood lymphocytes. Fibroblasts, B-cell lines, and leukemia T-cell lines bound 0.37–4.6 ng per $10^7$ cells. To determine whether nonmalignant T lymphoblasts expressed higher levels of p97 than normal peripheral blood lymphocytes, we also tested T cells purified from peripheral blood (0.03 ng per $10^7$ cells) and phytohemaglutinin-induced T lymphoblasts (0.00 ng per $10^7$ cells). The mouse lymphoma LSTRA, used as a negative control, bound <0.005 ng per $10^7$ cells.

Table 1. Fab 96.5 binding assay of cultured cells and peripheral blood lymphocytes

| Cells | Competitor | $^{125}$I-Labeled Fab 96.5, ng/$10^7$ cells | $^{131}$I-Labeled normal Fab, ng/$10^7$ cells |
|---|---|---|---|
| SK-MEL 28 melanoma* | | 490 | ND |
| KZ2 melanoma† | | 197 | 0.01 |
| 1799 melanoma‡ | | 170 | ND |
| 1688 melanoma‡ | | 63 | ND |
| 925 breast carcinoma‡ | | 11 | ND |
| KZ2 fibroblast† | | 1.2 | 0.02 |
| KZ2 fibroblast | Fab 96.5 | 0.04 | 0.02 |
| KZ2 B cell line§ | | 4.6 | 0.03 |
| GP B cell line§ | | 2.4 | 0.02 |
| GP B cell line | Fab 96.5 | 0.01 | 0.01 |
| GP B cell line | Ab 4.1 | 0.01 | 0.00 |
| GP B cell line | Ab 96.6 | 2.2 | 0.01 |
| Jurkat T leukemia§ | | 0.13 | 0.01 |
| Molt 4 T leukemia§ | | 1.5 | 0.01 |
| CEM T leukemia§ | | 0.83 | 0.02 |
| 8402 T leukemia§ | | 0.37 | 0.02 |
| NALM/6 ALL§ | | 0.18 | 0.01 |
| Lymphocytes‡ | | 0.06 | 0.00 |
| Lymphocytes | Fab 96.5 | 0.00 | 0.00 |
| LSTRA mouse lymphoma¶ | | 0.00 | 0.00 |

* Cells obtained from Sloan–Kettering Cancer Research Center, New York.
† Cells obtained from P. Wright (Fred Hutchinson Cancer Research Center, Seattle, WA).
‡ Cells originated in our own laboratory.
§ Cells obtained from J. Hansen (Histocompatibility Laboratory, Puget Sound Blood Center, Seattle, WA).
¶ Cells obtained from A. Fefer (University of Washington, Seattle, WA).

To evaluate the significance of the low level of binding of Fab 96.5 to non-melanoma cells, we used two controls. First, the cells were tested (in the same incubation mixture) with $^{131}$I-labeled Fab fragments prepared from normal mouse $IgG_2$. As shown in Table 1, binding was in each case less than 0.03 ng per $10^7$ cells. Second, we tested cells that had been preincubated with 10–20 μg of unlabeled antibody or Fab as competitor. Fab 96.5 and antibody 4.1 both completely blocked subsequent binding of $^{125}$I-labeled Fab 96.5. Neither antibody 96.6, which recognizes a different melanoma antigen (2), nor normal $IgG_2$ gave significant inhibition.

**DDIA for p97.** In order to measure p97 concentrations in membrane lysates prepared from cultured cells and tissue samples, we developed a new, highly sensitive, and specific assay, referred to as DDIA. DDIA utilizes two monoclonal antibodies

Table 2. p97 DDIA of cultured cells

| Melanomas and carcinomas | Fab 96.5,* ng/mg | Other cells | Fab 96.5,* ng/mg |
|---|---|---|---|
| SK-MEL 28 melanoma | 270 | AS/FG HEK 293-1† | 4.8 |
| 1688 melanoma‡ | 170 | LN fibroblasts† | 3.4 |
| KZ2 melanoma§ | 140 | GP fibroblasts‡ | 2.7 |
| KZ24 melanoma§ | 62 | Flow fibroblasts† | 1.6 |
| 1804 melanoma‡ | 60 | LN/SV40† | 1.4 |
| 1801 melanoma‡ | 58 | KZ2 fibroblasts§ | 0.3 |
| 992 kidney carcinoma‡ | 21 | 1553 mouse bladder carcinoma‡ | 0.0 |
| 907 bladder carcinoma‡ | 14 | MC55 transf. mouse fibroblasts¶ | 0.0 |

* $^{125}$I-Labeled.

† Cells obtained from J. MacDougal (Fred Hutchison Cancer Research Center, Seattle, WA): adenovirus 5-transformed human embryonic kidney cells, skin fibroblasts from a Lesch–Nyhan patient, Flow fibroblasts, and LN fibroblasts transformed by simian virus 40.

‡ Cells originated in our own laboratory.

§ Cells obtained from P. Wright (Fred Hutchinson Cancer Research Center, Seattle, WA.

¶ Cells obtained from A. Sen (National Cancer Institute).

to two different antigenic determinants of p97. One antibody (118.1) is used to quantitatively bind the p97 in the test sample to a solid phase (formalin-fixed *S. aureus*), and the amount of antigen bound is then measured by using radiolabeled antibody ($^{125}$I-labeled Fab 96.5) to the second determinant.

In a typical experiment, 20 μl of a lysate of membranes from melanoma biopsy 2161 (830 μg of protein per ml, or 16.6 μg total) was tested. Bound $^{125}$I-labeled Fab 96.5 measured 38,500 cpm. When the Tris/NaCl/EDTA/Nonidet P-40 buffer was tested as negative control, binding measured 109 cpm, and when antibody 118.1 was omitted from the first step of the assay, binding measured 92 cpm. The specific radioactivity of the $^{125}$I-labeled Fab 96.5 was 29,800 cpm/ng, giving 1.29 ng of Fab 96.5 bound or 78 ng of Fab 96.5 per mg of membrane protein. When 20 μl of a 1:10 dilution of the same lysate was tested, 3810 cpm were measured as bound, which corresponded to 0.124 ng of Fab 96.5 over background or 75 ng of Fab 96.5 per mg of protein, showing the linearity of the DDIA in the range 0–1 ng of Fab 96.5 bound.

**p97 DDIA of Cultured Cells.** Table 2 shows the results obtained when membrane lysates from cultured cells were tested by DDIA. The amount of Fab 96.5 bound ranged from 270 ng/mg for SK-MEL 28 melanoma to 0.3 ng/mg for KZ2 fibroblasts. Mouse cells did not bind detectable amounts of Fab 96.5 (<0.05 ng/mg).

**p97 DDIA of Tissues.** In addition to testing cultured cells, we studied a large number of tissue samples (Tables 3 and 4). The amount of Fab 96.5 bound ranged from 0.1–610 ng/mg of membrane protein, the highest binding being seen with melanomas, some other tumors, and certain fetal tissues. For normal tissues, the amount bound ranged from 0.1–10 ng/mg, the highest binding being seen with vagina and uterus tissues. The significance of the low levels of binding to the normal adult tissues was established by running several controls. The normal adult liver lysate, which bound 1.2 ng/mg, was used for this purpose. The first control was to pretreat 60 μl of the liver lysate with 1 μg of antibody 96.5 and 5 mg of *S. aureus* in order to remove p97 before the DDIA. In the second control, antibody 118.1 was omitted from the first step of the DDIA. The third control was to use $^{125}$I-labeled normal Fab as the labeled probe. The fourth control was to add 7 μg of unlabeled Fab 96.5 in the second step of the assay as competitor. Finally, mouse cells, which we expected to contain no p97, were tested. All of these controls were negative—less than 0.02 ng of Fab binding per mg of protein. Therefore, the binding obtained with the normal liver lysate (1.2 ng/

Table 3. p97 DDIA of tumors

| Melanomas | Fab 96.5,* ng/mg | Other tumors | Fab 96.5,* ng/mg |
|---|---|---|---|
| 2178 melanoma | 610 | 2192 adenomyosis | 41 |
| 2207 melanoma | 240 | JL1 benign nevus | 30 |
| KZ52 melanoma | 190 | 2206 liposarcoma | 28 |
| 1801 melanoma | 150 | 2195 leiomyosis | 20 |
| 2161 melanoma | 75 | 2214 breast carcinoma | 10 |
| 2169 melanoma | 75 | 2185 lung carcinoma | 3.7 |
| KZ53 melanoma | 45 | RC lung carcinoma | 2.8 |
| AH melanoma | 35 | AH benign nevus | 0.1 |
| 2210 melanoma | 28 | LS kidney carcinoma | 0.1 |
| KZ51 melanoma | 27 | 2148 kidney carcinoma | 0.1 |
| 2102 melanoma | 13 | | |
| 2196 melanoma | 6.8 | | |
| 2184 melanoma | 2.8 | | |
| 2141 melanoma | 2.7 | | |
| GP melanoma | 2.2 | | |
| 2189 melanoma | 2.2 | | |
| JM melanoma | 2.0 | | |
| 1817 melanoma | 0.1 | | |

* $^{125}$I-Labeled.

Table 4. p97 DDIA of normal tissues

| Adult tissues | Fab 96.5,* ng/mg | Fetal tissues | Fab 96.5,* ng/mg |
|---|---|---|---|
| Uterus | 10 | Colon | 110 |
| Vagina | 7.9 | Umbilical cord | 31 |
| Bladder | 5.3 | Heart | 14 |
| Muscle | 4.1 | Kidney | 2.4 |
| Ovary | 2.3 | Lung | 2.2 |
| Colon | 2.2 | Brain | 2.1 |
| Ileum | 1.3 | Tongue | 0.2 |
| Liver | 1.2 | Eye | 0.1 |
| Kidney | 1.1 | | |
| Lung | 0.9 | | |
| Cerebellum | 0.6 | | |
| Cerebrum | 0.5 | | |
| Stomach | 0.5 | | |
| Placenta | 0.4 | | |
| Skin | 0.4 | | |
| Substantia nigra | 0.4 | | |

* $^{125}$I-Labeled.

mg) was real and, by inference, the binding obtained with the other normal tissues was real also.

**p97 DDIA of Serum.** Normal sera and sera from melanoma patients were assayed. Normal sera from nine individuals were found to bind 1.3–2.7 ng of Fab 96.5 per ml (median 1.8). Of 13 melanoma patients tested, 11 had sera with values that were in the same range. However, sera from two patients bound higher levels (3.6 and 5.4 ng/ml).

## DISCUSSION

Melanomas and some other human tumors express p97 at the cell surface, as we have demonstrated both by binding assays of cultured cells with radiolabeled antibody 4.1 and by immunoprecipitation from lysates of radiolabeled membranes prepared from tumor biopsies (1). A critical question is whether p97 is expressed by normal cells. Previous results indicated that normal adult cells expressed very little p97, if any (1).

The first suggestion that p97 might be more widely distributed came from borderline positive results of binding assays of fibroblasts and B-cell lines and from a very small amount of p97 detected by immunoprecipitation in one fibroblast culture. We decided to investigate this more closely. In order to lower the background of the binding assay, we used another monoclonal antibody, 96.5, rather than the antibody 4.1 used in the original study (1). Antibody 96.5 has a very high affinity for p97 (approximately $10^{10}$ $M^{-1}$, data not shown). The possibility of artifactual binding to cells through the Fc region of the antibody was eliminated by using Fab fragments; more than 50% of a $^{125}$I-labeled Fab 96.5 preparation was able to bind to cultivated SK-MEL 28 melanoma cells. To make the assay more sensitive, we tested more cells (up to $2 \times 10^6$) than previously (1) and minimized nonspecific binding by using fetal calf serum as diluent, incubating the cells at 0°C, and washing the cells more thoroughly. As shown in Table 1, all the human cells tested bound $^{125}$I-labeled Fab 96.5, although melanoma cells bound much more (up to 490 ng per $10^7$ cells) than, for example, fibroblasts (1 ng per $10^7$ cells), B-cell lines (about 4 ng per $10^7$ cells), or peripheral blood lymphocytes (0.06 ng per $10^7$ cells).

The demonstration of small amounts of p97 on normal cells prompted us to reexamine normal tissues for expression of p97. Immunoprecipitation tests of normal adult tissues had been negative (1), but it was apparent that this technique was not capable of detecting amounts of p97 as small as those observed in the experiments listed in Table 1. The binding assay with radiolabeled Fab 96.5 was not suitable for testing tissues; a method of measuring p97 in tissue lysates was needed.

The isolation of a hybridoma, 118.1, that secretes an antibody to a second antigenic determinant of p97 ($p97^b$) enabled us to develop a new immunoassay method, which we have termed "double-determinant immunoassay." DDIA offers a new dimension in both immunological specificity and sensitivity. Given identical antibody affinities, DDIA is much more sensitive than competition radioimmunoassay because the antigen can be concentrated in the first step and because one measures the antigen directly rather than by competition. DDIA is particularly valuable for antigens identified by monoclonal antibodies because in many cases purified antigen is not available for use in competition radioimmunoassay, whereas pure, monospecific antibody can be readily prepared.

The p97 DDIA was used by us to test a panel of normal adult, fetal, and neoplastic tissues. All of the tissues tested contained detectable amounts of p97. As expected from our previous work (1), melanomas contained the most p97, binding up to 610 ng/mg. Several fetal tissues bound a considerable amount (colon, 130 ng/mg; umbilical cord, 31 ng/mg). Other tumors, both malignant (kidney carcinoma, 21 ng/mg) and benign (nevus, 30 ng/mg; tissue from adenomyosis, 41 ng/mg; and leiomyoma, 20 ng/mg) bound high levels. Normal adult tissues bound 0.1–10 ng/mg.

In evaluating the results of both the binding assays of intact normal cells (such as peripheral blood lymphocytes) and the DDIA of normal tissue lysates, an important question is whether the binding of $^{125}$I-labeled Fab 96.5 is actually due to the presence of small amounts of p97 or whether it is artifactual. With respect to the binding assays, our specificity controls indicate that the Fab 96.5 does not bind nonspecifically to cells because very little normal Fab bound, and the binding of Fab 96.5 could be competed only with antibodies specific for p97—namely, 4.1 and 96.5. Moreover, Fab 96.5 did not bind to mouse cells. The monoclonal nature of the Fab 96.5 eliminates the possibility of contaminating specificities, such as might be found in an antiserum. The question remains whether the Fab 96.5 might be binding to a protein other than p97 [but with either the same ($p97^a$) or a cross-reactive antigenic determinant] either fortuitously or because of its being structurally related. An immunoprecipitation test of one fibroblast line, GP, with antibody 96.5 revealed a faint band corresponding to 97 kilodaltons, which argues against the first possibility but not the second (because a protein related to p97 might be expected to have a similar molecular size).

With regard to the assays of solubilized preparations, we can come to more definite conclusions. To be detected in the p97 DDIA, a protein must have two antigenic determinants ($p97^a$, $p97^b$) or strongly crossreactive determinants. Because either determinant alone is enough to distinguish p97 from hundreds of other cell-surface proteins in melanoma cells (as shown by our seeing only one 97 kilodalton species in immunoprecipitation assays), the chance of an unrelated protein fortuitously having both is infinitesimal. We conclude that, in fact, normal tissues do contain low levels of p97.

The two highly sensitive assays that we have used enabled us to demonstrate very low concentrations of p97 in normal tissues and serum—concentrations too low to be detected by methods used previously to examine tumor antigens. Carcinoembryonic antigen and $\alpha$-fetoprotein, for which competition radioimmunoassays are available, are exceptions (8, 9); in fact, these antigens can be detected in normal adults (10, 11). We consider that p97, like carcinoembryonic antigen and $\alpha$-fetoprotein, is most appropriately described as an oncofetal antigen because it is present in large amounts only in tumors and fetal tissues. Whether the quantitative differences between p97 levels in various normal adult tissues indicate that p97 also may be associated with a particular differentiated cell type is unclear for several reasons. First of all, for most of the normal tissues, only a single sample was obtained. These samples varied (*i*) in origin (surgery or autopsy), (*ii*) in the reason for surgery or the cause of death, and (*iii*) in the handling of the sample subsequently (such as time of autopsy after death, time the sample was kept on ice before freezing, and preparation of the membrane lysate). The ability to localize p97 at a microscopic level in tissues, for instance by immunofluorescence or immunoperoxidase methods, appears to offer a better chance of determining whether p97 is associated with a particular normal cell type than further assays of tissue lysates.

Monoclonal antibodies allow much better definition of tumor antigen specificity than has been possible (12). Furthermore, highly sensitive and quantitative assays, such as those that we have used, are made possible. The availability of specific and sensitive analytical techniques means that tumor antigens can (and probably also should) be defined in quantitative terms. The particular tumor antigen that we have studied, p97, is present

in many melanomas in 100-fold greater concentrations than in most normal adult tissues. We believe that this difference is sufficiently large that monoclonal antibodies to p97 may prove to be of clinical value. First, monoclonal antibody to p97 might be used for radionuclide imaging studies for detection of metastases (13). If labeled anti-p97 antibody can be shown to concentrate in melanomas *in vivo*, such antibody also may be useful for delivering therapeutic agents to tumor metastases (14); of course, proper precautions would be needed to investigate this, particularly if the contemplated microscopic assays will demonstrate large amounts of p97 in some normal stem cell populations. Whether DDIA of p97 in serum will prove to be of value diagnostically is more questionable, because normal serum contains significant amounts of p97, elevated levels being seen in only a few melanoma patients with advanced disease. It is possible, however, that measurement of circulating p97 may be useful for monitoring selected patients with large tumor burdens.

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