

# Binding of Serum Albumin on Tumor Cells and Characterization of the Albumin Binding Protein

Jue Wang, Hironao Ueno, Takashi Masuko, and Yoshiyuki Hashimoto<sup>1</sup>

Department of Hygienic Chemistry, Pharmaceutical Institute, Tohoku University, Aramaki Aza Aoba, Aoba-ku, Sendai 980

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The binding of bovine (BSA) and human serum albumin (HSA) to the surface of human and mouse tumor cells *in vitro* was examined by flow cytometry using monoclonal antibodies against these albumins. Both isologous and heterologous types of albumin bound firmly on several lines of human and mouse tumor cells. The cell-bound albumin was removable by protease (actinase) treatment or by culture in albumin-free medium for more than 2 days, but not by simple washing. The amount of albumin capable of binding to the tumor cell surface differed among the 9 tumor cell lines tested. As determined with radioiodinated BSA, about  $2.6 \times 10^6$  BSA molecules/cell could bind to MDA-MB-453 human mammary cancer cells, which exhibit high binding capacity as to BSA. A unique peptide having a molecular weight of 18 kDa was detected on gel electrophoresis in extracts of tumor cells or normal aortic endothelial cells which had been treated with radioiodinated, photocross-linker-labeled BSA or HSA, followed by UV-irradiation. This peptide was also immunoprecipitated with an anti-BSA or anti-HSA monoclonal antibody. With both methods, the yield of the peptide was decreased by previous addition of an excess amount of unlabeled BSA or HSA. These findings indicated that the 18-kDa peptide expressed on both normal aortic endothelial cells and tumor cells is a principal serum albumin-binding protein, and that this protein binds both BSA and HSA.

**Key words:** albumin binding protein, bovine serum albumin, human serum albumin, tumor cells.

Albumin was found to bind with cultured endothelium and a variety of tissues *in situ* (1-3) through albumin binding proteins (ABPs) (4-8). Studies on the binding and characterization of ABPs have been mainly performed using normal endothelial cells, *i.e.*, little with other types of cells, including neoplastic cells.

To determine whether or not albumin is also able to bind to neoplastic cells and whether or not neoplastic cells express an albumin binding protein, we analyzed the binding of BSA and HSA on various types of human and mouse tumor cells and examined the presence of albumin-binding proteins in the tumor cells. Here, we report that tumor cells can bind not only heterologous albumin but also isologous albumin on their surface with the aid of an ABP.

## MATERIALS AND METHODS

**Chemicals**—Fetal calf serum (FCS) was obtained from

Flow Laboratories, Rockville, MD, and heat-inactivated at 56°C for 30 min before use. Bovine serum albumin (BSA, Fraction V) and human serum albumin (HSA, Fraction V) were purchased from Sigma Chemical, St. Louis, MO. Fluorescein isothiocyanate (FITC)-conjugated BSA was a generous gift from Dr. K. Ohuchi, Department of Biochemistry of our institute. Serum-free medium, Hymedium 606, was purchased from Kojin, Tokyo. Dulbecco's modified Eagle's medium (DMEM) was obtained from Nissui Pharmaceutical, Tokyo, and was supplemented with 2 mM L-glutamine, 1  $\mu$ M sodium pyruvate, 10 mM HEPES, kanamycin at 60  $\mu$ g/ml, and penicillin at 100 U/ml. Na<sup>125</sup>I was purchased from Amersham Lab., Buckinghamshire, England, skimmed milk from Yukijirushi, Tokyo, and iodogen and sulfosuccinimidyl 2-(*p*-azidosalicylamido)ethyl-1,3'-dithiopropionate (SASD) from Pierce, Rockford, IL.

**Monoclonal Antibodies (mAbs)**—Two anti-BSA mAbs (AB-3 and AB-6), which recognize different epitopes on the BSA molecule, and HBJ127 mAb, which recognizes human gp125 cell surface antigen (9), were produced in syngeneic mice by transplantation of the corresponding hybridomas prepared in our laboratory. Antibodies were purified from ascitic fluid of the mice by 50% saturated ammonium sulfate precipitation followed by DEAE column chromatography. For flow cytometric analysis, each mAb was dissolved in PBS containing 0.5% skimmed milk and used at the concentration of 20  $\mu$ g/ml. The mouse anti-HSA mAb,

<sup>1</sup> To whom correspondence should be addressed.

Abbreviations: ABP, albumin binding protein; ASD, 2-(*p*-azidosalicylamido)ethyl-1,3'-dithiopropionate; BAEC, bovine aortic endothelial cells; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; HSA, human serum albumin; mAb, monoclonal antibody; MFI, mean fluorescence intensity; PBS, phosphate-buffered saline, pH 7.4; SASD, sulfosuccinimidyl 2-(*p*-azidosalicylamido)ethyl-1,3'-dithiopropionate; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis.

AH-1, was obtained from Medix Biochemica, Finland, and used after appropriate dilution.

**Cells**—The cells used were human breast cancer (MDA-MB-453, SK-Br-3, MCF-7, and YMB-1-E), stomach cancer (KATO-III), and leukemia (K562 and Molt-4F) cell lines, and mouse Meth-A fibrosarcoma and P3X63Ag8.653 (X63) ones. All these cell lines were maintained by serial culture in DMEM medium containing 10% FCS.

Bovine (BAEC) and human aortic endothelial cells (HAEC) were kindly donated by Drs. K. Ohuchi and Y. Suzuki of our institute, respectively, and used after 4 or 5 passages. Cells harvested after culture were washed once with PBS and then cultured in Hymedium 606 for 2 days before use.

**Preparation of BSA-Free Tumor Cells**—To remove BSA from tumor cells which had been cultured in FCS-containing medium, the tumor cells were washed once with PBS, and then treated for 5 min at 37°C with PBS containing actinase at 7.5 ng/ml and 0.05% EDTA (pH 7.4). The absence of BSA on the tumor cells was confirmed by indirect immunofluorescence (see below), and then the BSA-free tumor cells were cultured in Hymedium 606 (serum free) medium and used for experiments.

**Analysis of BSA and HSA Bound on the Tumor Cell Surface**—BSA-free tumor cells ( $2 \times 10^5$ ) were cultured in DMEM containing 10% FCS, washed 3 times with PBS, and then suspended in PBS containing 0.5% skimmed milk. For the indirect immunofluorescence assay, an aliquot (100  $\mu$ l containing  $2 \times 10^5$  cells) of a cell suspension was treated with a solution of the relevant mAb solution (100  $\mu$ l) for 1 h at 4°C, and then the cells were washed twice with PBS and stained with 100  $\mu$ l of 1:200 diluted FITC-conjugated rabbit anti-mouse Ig for 1 h at 4°C. After two washes, the cells were analyzed for cell-surface fluorescence with a FACScan (Becton Dickinson). For the direct fluorescence assay, BSA-free cells were cultured in medium containing 10% FITC-BSA and then total fluorescence was analyzed with a FACScan. The fluorescence intensity was expressed as the mean fluorescence intensity (MFI).

To examine the binding of HSA on the cell surface, BSA-free tumor cells were cultured for 48 h in Hymedium 606 containing 10% HSA. The amount of HSA bound on the cell surface was determined as described above using anti-HSA mAb.

**Analysis of BSA Molecules Capable of Binding to a MDA-MB-453 Tumor Cell**—The number of BSA molecules capable of binding to a MDA-MB-453 tumor cell was determined as to direct binding of  $^{125}$ I-labeled ( $^{125}$ I-)BSA and as to binding of  $^{125}$ I-labeled AB-3 mAb to tumor cells which had been treated with a saturable amount of BSA. BSA or AB-3 mAb was labeled with  $^{125}$ I by the iodogen method as described (10), and then diluted with an appropriate amount of the unlabeled material. For direct binding of  $^{125}$ I-BSA,  $5 \times 10^6$  cells were cultured for 24 h at 37°C in the presence of  $^{125}$ I-BSA ( $1.3 \times 10^6$  cpm/tube), washed three times with PBS, and then subjected to radioactivity determination using a  $\gamma$ -ray scintillation counter (Aloka, Tokyo). For analysis with  $^{125}$ I-mAb, BSA-free MDA-MB-453 cells were pretreated with BSA (1 mg or 10 mg/ml) and then washed four times with PBS. An aliquot of the cells ( $2 \times 10^6$ ) was then incubated for 6 h at 4°C in the presence of various amounts of the  $^{125}$ I-AB-3 mAb (specific activity,  $1.8 \times 10^4$  cpm/ng) in PBS containing 0.1% gelatin

and 0.01 M HEPES. The cells were washed three times with PBS and then the residual radioactivity on cells was determined with a  $\gamma$ -ray scintillation counter. To compensate for the nonspecific binding of labeled mAb, the target cells were incubated with labeled mAb in the presence of an excess amount of unlabeled mAb (800-fold the amount of the labeled mAb), and the radioactivity remaining on the cells was subtracted from the above value. All these experiments were performed in duplicate and average values were obtained.

**Detection of ABP by Cross-Linking of Albumin with the Tumor Cell Surface Protein**—Preparation of a radiolabeled cross-linker,  $^{125}$ I-SASD, its coupling to albumin, and conjugation of  $^{125}$ I-SASD-modified albumin ( $^{125}$ I-ASD-albumin) to cells were performed as described by Ghinea *et al.* (11). The specific radioactivities of  $^{125}$ I-ASD-BSA and  $^{125}$ I-ASD-HSA were 0.85 and 0.86  $\mu$ Ci/ $\mu$ g, respectively.

The target cells were pretreated with 2% polyvinylpyrrolidone in PBS for 1 h at 22°C to prevent nonspecific binding of albumin, and then an aliquot of the cell suspension ( $4 \times 10^6$  cells) was treated with 150  $\mu$ l of a  $^{125}$ I-ASD-albumin solution (60  $\mu$ g of albumin) for 1 h in a dark room at room temperature, washed three times with PBS, and finally resuspended in PBS. The cell suspension was placed in an ice bath and then exposed to UV-light (312 nm, 15 W lamps  $\times 2$ ) for 6 min at a distance of 5 cm. After two washes with PBS, the cells were lysed with 150  $\mu$ l of Tris-buffered saline (pH 7.4) containing 0.5% Nonidet P-40 (NP-40) and 0.1 mM (*p*-amidinophenyl)methanesulfonyl fluoride for 2 h at 4°C. After centrifugation, 50  $\mu$ l of each cell lysate was subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The gels were dried in vacuum and then radioactive protein bands were detected by autoradiography using an X-ray film.

For control experiments, cells were incubated with  $^{125}$ I-ASD-albumin in the presence of a 300-fold excess of unlabeled albumin as a competitor or cells with bound  $^{125}$ I-ASD-albumin were processed without UV-irradiation.

**Immunoprecipitation**—Affinity gel modified with anti-BSA mAb (AB-6) was prepared by coupling Cellulofine (Seikagaku Kogyo, Tokyo) with an mAb in 0.1 M carbonic buffer, pH 8.0. An aliquot of each cell lysate (20  $\mu$ l) was incubated with 20  $\mu$ l of the affinity gel overnight at 4°C. After five washes with 0.05% Tween-PBS, the gel was boiled for 5 min in 20  $\mu$ l of sample buffer containing 10% 2-mercaptoethanol and then the supernatant was analyzed by SDS-PAGE as described above.

## RESULTS

**Binding of BSA to Human and Mouse Tumor Cells**—Seven human and two mouse tumor cell lines were cultured in 10% FCS-containing medium for 7 days, and then well-washed cells were analyzed for cell-bound BSA by flow cytometry using AB-3 and AB-6 mAb against BSA. Representative FACS patterns and the relative amounts of BSA bound on the tested tumor cells are shown in Fig. 1 and Table I, respectively. The amount of BSA bound on the cell surface differed with the cell line and was not related to the tissue type: The amount of bound BSA was relatively high for MDA-MB-453 and X63 cells, intermediate for SK-Br-3, MCF-7, and K562 cells, and low for YMB-1-E, KATO-III, Molt-4, and Meth-A cells. Based on the results, human

MDA-MB-453 breast cancer cells were mainly used in the following experiments.

The BSA bound on the cell surface was removed completely by actinase treatment but not by repeated washing with PBS (Fig. 2). When MDA-MB-453 cells precultured in 10% FCS-containing medium were transferred to serum-free Hymedium 606 medium and cultured, cell-bound BSA was gradually released from the cells, and after 48 h the cells were almost completely free from BSA, as assessed by flow cytometry (Fig. 3). This was not due to integration of the bound albumin into cells, as judged on immunostaining of formalin-fixed cells (data not shown).

**Dependence of BSA Binding on the Culture Period, Temperature, and Concentration of BSA**—BSA-free MDA-MB-453 cells precultured in Hymedium 606 medium were transferred to the medium supplemented with 10% FCS or 10 mg/ml of BSA. Cells were periodically harvested and analyzed for the binding to BSA by flow cytometry (Fig. 4). In cultures in both FCS- and BSA-containing medium at 37°C, the amount of BSA bound on cells increased until 24 h and then reached a plateau level (Fig. 4A). When tumor cells were incubated for 24 h at 4°C, BSA did not bind to the cells (Fig. 4B). This was in contrast to regular antibody-antigen binding which takes place at either 37 or 4°C, as observed with the binding of HBJ127 mAb to cells (see Fig. 4B).

TABLE I. Reactivity of anti-BSA mAbs with various tumor cell lines.

| Origin           | Cell line  | Species | MFI <sup>a</sup> of cells stained with |      |
|------------------|------------|---------|----------------------------------------|------|
|                  |            |         | AB-3                                   | AB-6 |
| Carcinoma Breast | MDA-MS-453 | Human   | 15.9                                   | 15.3 |
|                  | SK-Br-3    | Human   | 4.2                                    | 3.9  |
|                  | MCF-7      | Human   | 2.0                                    | 4.4  |
|                  | YMB-1-E    | Human   | 0.9                                    | 1.6  |
| Stomach          | KATO-III   | Human   | 0.7                                    | 0.7  |
| Leukemia         | K562       | Human   | 4.9                                    | 4.0  |
|                  | Molt-4F    | Human   | 0.6                                    | 0.5  |
| Fibrosarcoma     | Meth-A     | Mouse   | 1.1                                    | 0.8  |
| Myeloma          | X63        | Mouse   | 14.4                                   | 9.2  |

<sup>a</sup>Cells were cultured in medium containing 10% FCS for more than 7 days after subculture. MFI values shown were obtained by subtraction of control (without first antibody) values from experimental ones.

The relationship between the concentration of BSA in the culture medium and the amount of BSA bound on the cell surface was assessed by flow cytometry using indirect or direct immunostaining of MDA-MB-453 cells. The BSA-

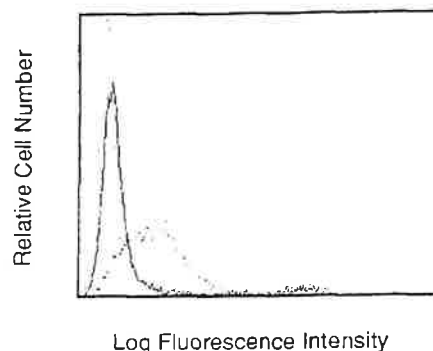


Fig. 2. Effect of actinase treatment on the amount of BSA bound on MDA-MB-453 cells. Cells were cultured in medium containing 10% FCS and then washed. Before or after treatment with actinase E, cells were stained by indirect immunofluorescence using AB-6 mAb. —, after actinase E treatment; ----, before actinase treatment; ····, negative control without first antibody.

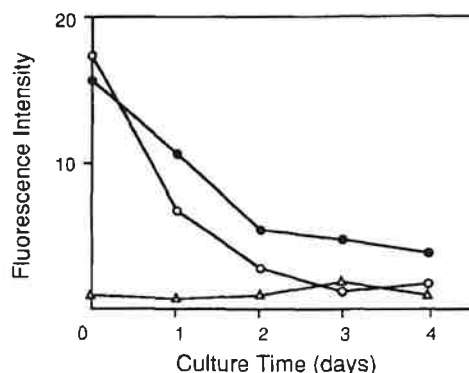


Fig. 3. Release of surface-bound BSA from MDA-MB-453 cells on culture in serum-free medium. Cells were cultured in medium containing 10% FCS, washed, and then cultured in Hymedium 606 medium. ○, cells stained using AB-3 mAb; ●, cells stained using AB-6 mAb; △, after actinase E treatment, cells were cultured in serum-free medium and then stained with AB-3 mAb.

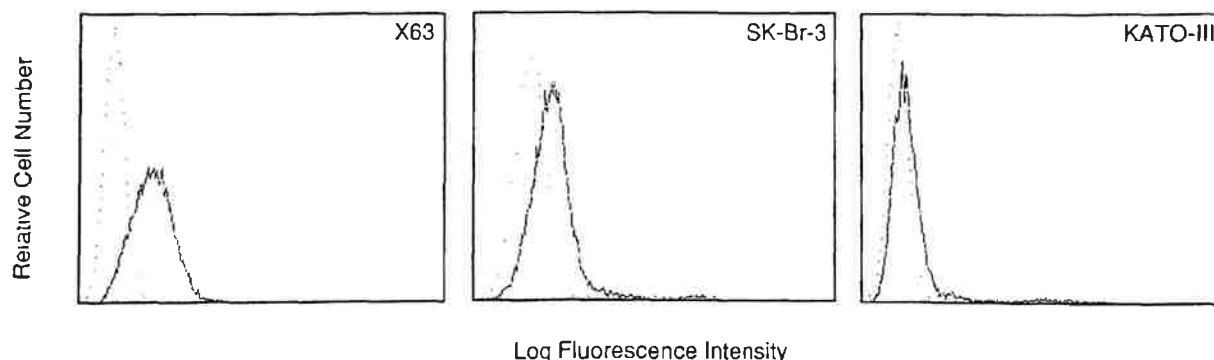


Fig. 1. BSA molecules bound to the surface of cells cultured in 10% FCS-containing medium. —, cells stained by indirect immunofluorescence using AB-6 mAb; ····, negative control without first antibody.

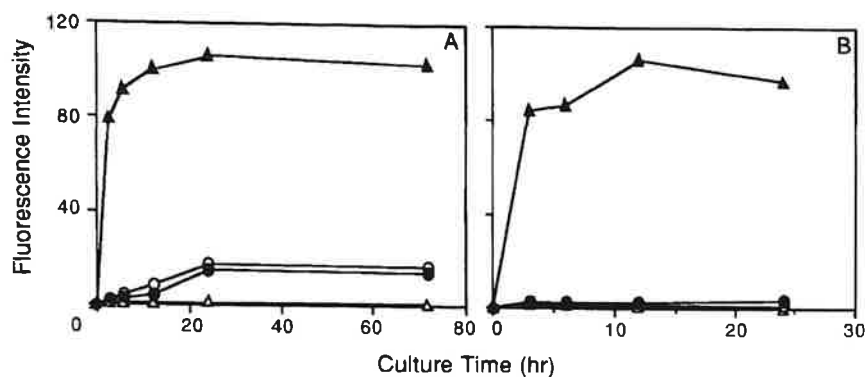


Fig. 4. Effect of temperature on the binding of BSA and mAb against gp125 antigen to MDA-MB-453 tumor cells. MDA-MB-453 cells were cultured in medium containing BSA at 10 mg/ml or 10% FCS and then stained by indirect immunofluorescence, and then the binding of BSA was assessed by flow cytometry. ●, cells cultured in BSA-medium and then stained with AB-6 mAb; ○, cells cultured in FCS-medium and then stained with AB-6 mAb; ▲, cells cultured in FCS-medium and then stained with anti-gp125 (HBJ127) mAb; △, after treatment with actinase E, cells were cultured in serum-free medium and then stained with AB-6 mAb. A, at 37°C; B, at 4°C.

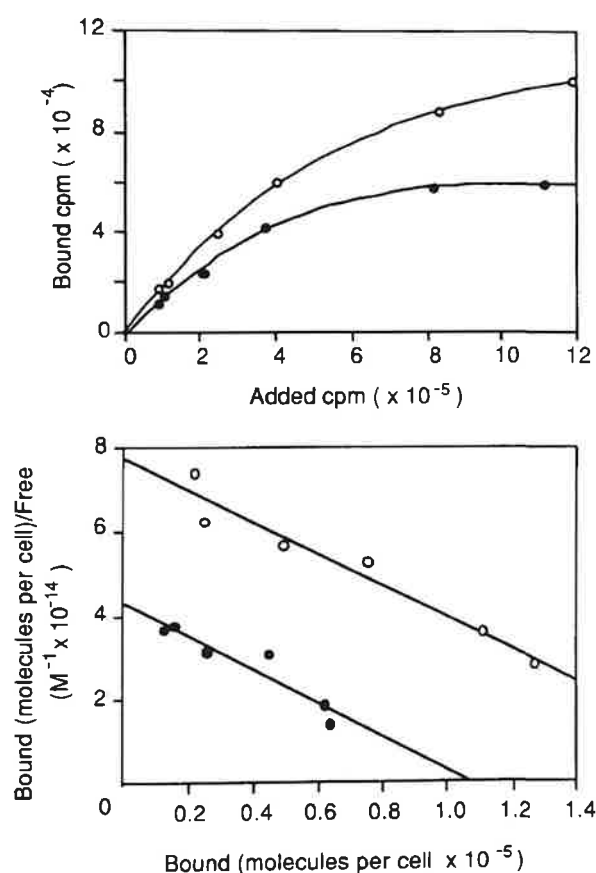


Fig. 5. Scatchard plot analysis of the number of BSA molecules capable of binding on the surface of MDA-MB-453 cells. BSA-free MDA-MB-453 cells were cultured in Hymedium 606 in the presence of BSA and then the amount of BSA-bound cells was assayed as described under "MATERIALS AND METHODS." ○, BSA at 10 mg/ml; ●, at 1 mg/ml.

binding on cells was dependent on the concentration of BSA in the medium and reached a plateau at a BSA concentration of 5 mg/ml (data not shown).

**Number of BSA Molecules Capable of Binding to a Tumor Cell**—BSA-free MDA-MB-453 cells ( $5 \times 10^5$ ) were cultured for 24 h at 37°C in the presence of  $^{125}\text{I}$ -BSA ( $1.3 \times 10^6$  cpm/mg) and unlabeled BSA, and then washed three

TABLE II. Binding of HSA on the tumor cell surface.

| Target cells | MIF <sup>a</sup> of cells bound with |            |
|--------------|--------------------------------------|------------|
|              | HSA (AH-1)                           | BSA (AB-3) |
| MDA-MB-453   | 14.9                                 | 8.4        |
| K562         | 7.0                                  | 6.6        |
| X63          | 13.4                                 | 14.5       |
| Meth-A       | 5.2                                  | 1.1        |

<sup>a</sup>BSA-free tumor cells were cultured in the presence of HSA or BSA (10 mg/ml) for 24 h at 37°C, and then MIF was determined as to indirect immunofluorescence using AH-1 or AB-3 mAb.

times with PBS. The radioactivity of the cells incubated with  $^{125}\text{I}$ -BSA at 10 mg/ml (a saturable amount for cell binding) was  $1.8 \times 10^4$  cpm. This result indicated that BSA in the saturable amount bound to MDA-MB-453 cells at  $2.6 \times 10^5$  molecules/cell.

Cells cultured with unlabeled BSA (10 mg/ml) were washed three times with PBS and then treated with various amounts of  $^{125}\text{I}$ -AB-6 mAb for 6 h at 4°C. After washing, the cells were subjected to radioactivity counting, and the data were analyzed by means of Scatchard plots (Fig. 5). The results indicated that AB-6 mAb bound at  $2.03 \times 10^5$  molecules/cell to MDA-MB-453 cells with the saturable amount of BSA. Since AB-6 mAb binds with BSA in a molar ratio of about 1 : 1, as determined by ELISA (data not shown), about the same number of BSA molecules as that of the mAb ( $2 \times 10^5$ ) is suggested to be bound on the cells, this value being similar to that obtained with the radiolabeled BSA.

**Binding of HSA on Human Tumor Cells**—Since BSA is a heterologous protein as to target mouse and human tumor cells, we examined whether or not isologous albumin is also able to bind to tumor cells. BSA-free human or mouse tumor cells were cultured in Hymedium 606 containing HSA or BSA at 10 mg/ml. After the culture, the cells were washed with PBS and then analyzed for cell-bound albumin by use of anti-HSA or anti-BSA mAb (Table II). Significant binding of HSA was observed with both the human and mouse tumor cell lines tested. This finding indicated that isologous albumin (HSA) is also able to bind to human tumor cells.

**Detection of ABP by Cross-Linking with Radioactive Albumin**—After treatment with  $^{125}\text{I}$ -ASD-albumin, BAEC or MDA-MB-453 cells were exposed to UV light and then extracted with NP-40. The cell extracts were then subjected to SDS-PAGE under reduced conditions. Autoradio-

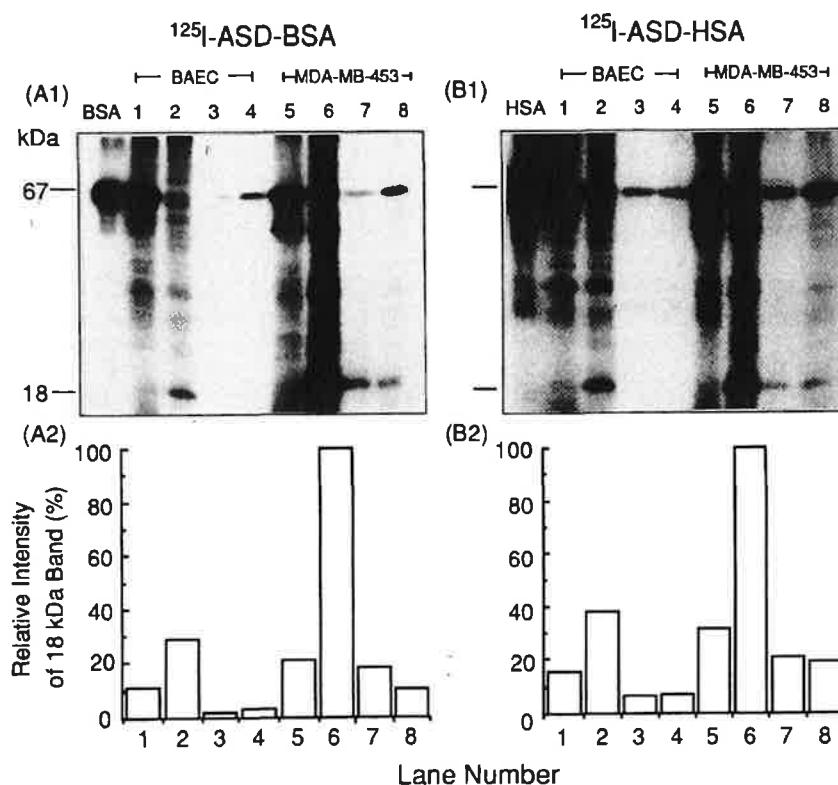


**Fig. 6. Detection of ABP by cross-linking with radioiodinated albumin.** BAEC (lanes 1–4) and MDA-MB-453 tumor cells (lanes 5–8) were treated with <sup>125</sup>I-ASD-BSA (A) and <sup>125</sup>I-ASD-HSA (B), respectively. After extensive washing, the cells were exposed to UV light and then extracted with NP-40. The extracts were subjected to SDS-PAGE and radioactive peptides were detected by autoradiography (lanes 2 and 6). Samples used as controls were extracts of cells treated with radioiodinated albumin in the presence of an excess amount of unlabeled BSA (lanes 3 and 7) or HSA (lanes 4 and 8), and extracts of cells treated without UV irradiation (lanes 1 and 5). The upper figures (A1 and B1) show the SDS-PAGE profiles, and the lower ones (A2 and B2) the relative radioactivity of each peptide band, corresponding to figures A1 and B1. Lane BSA, <sup>125</sup>I-ASD-BSA alone; lane HSA, <sup>125</sup>I-ASD-HSA alone.

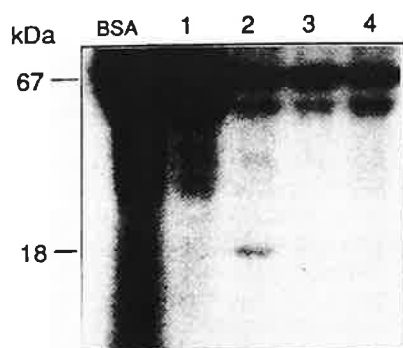


**Fig. 7. Detection of ABP by immunoprecipitation.** Extracts of MDA-MB-453 cells treated with <sup>125</sup>I-ASD-BSA and UV irradiation (see legend to Fig. 6) were immunoprecipitated by use of AB-6 mAb-modified affinity gel (lane 2). A UV-unirradiated sample (lane 1) and a sample treated in the presence of an excess amount of unlabeled BSA (lane 3) or HSA (lane 4) were used as controls.

grams of the gels and the density of each radioactive band are shown in Fig. 6. A peptide band corresponding to 18 kDa was observed for the photo-activated samples of both BAEC and MDA-MB-453 cells (Fig. 6, A and B, lanes 2 and 6), but much less for the UV-unirradiated samples (lanes 1 and 5). The 18-kDa peptide was also detected in similarly treated HAEC (data not shown). When cells were treated with <sup>125</sup>I-ASD-BSA in the presence of an excess amount of unlabeled BSA or HSA, the yield of the 18-kDa peptide was markedly reduced. A reduction of the amount of the 18-kDa peptide was also observed with the combination of

<sup>125</sup>I-ASD-HSA and unlabeled BSA or HSA (Fig. 6, A and B; lanes 3, 4, 7, and 8).

These findings indicated that the 18-kDa peptide is a major ABP, and that it is expressed on both normal aortic endothelial cells and human tumor cells.

**Detection of ABP by Immunoprecipitation**—To further examine the presence of ABP on tumor cells, extracts of MDA-MB-453 tumor cells, which had been treated with <sup>125</sup>I-ASD-albumin and UV-irradiation, were subjected to immunoprecipitation using the affinity gel coupled with AB-6 mAb, and the immunoprecipitated material was subjected to SDS-PAGE under reduced conditions (Fig. 7). A peptide of 18 kDa was immunoprecipitated as a characteristic component (lane 2), while this peptide was detected for neither the UV-unirradiated sample (lane 1) nor a sample treated in the presence of an excess amount of BSA or HSA (lanes 3 and 4).

## DISCUSSION

This work demonstrated that both isologous and heterologous albumins can bind to several lines of cultured tumor cells through a characteristic protein present on the cell surface. BSA bound firmly to either human or mouse tumor cells in such a manner that it was not removable by simple washing but depleted by culture in serum-free medium for more than 2 days or by proteolytic enzyme (actinase) treatment. The binding of albumin to the surface of tumor cells was saturable and selective, as judged by Scatchard plot analysis (Fig. 5), and by binding inhibition assays with the combination of a radiolabeled albumin (Figs. 6 and 7) or FITC-albumin (data not shown) and the unlabeled albumin.

The features of the binding of albumin to MDA-MB-453 tumor cells were basically in accordance with those for endothelial cells (3-7, 12).

Since albumin showed a certain degree of selectivity in its binding to tumor cells, it seems likely that tumor cells express albumin binding molecule(s) on their surface. When MDA-MB-453 tumor cells were successively treated with photocrosslinker-modified  $^{125}\text{I}$ -BSA and UV-irradiation, NP-40 extracts of the cells contained a characteristic radioactive peptide of 18 kDa, as determined by SDS-PAGE under reduced conditions. Furthermore, immunoprecipitation of the cell extracts with anti-BSA mAb also gave a peptide of 18 kDa on SDS-PAGE. Similar results were obtained by use of bovine aortic endothelial cells. Since this peptide was detected in neither unirradiated samples nor cells treated with a radioiodinated albumin in the presence of an excess amount of the unlabeled albumin, the 18-kDa peptide can be judged to be an ABP.

Besides the 18-kDa peptide, the presence of another ABP, a peptide of 31 kDa, on endothelial cells was detected on treatment of the blots of cell extracts with a radioiodinated or gold-labeled albumin (6-8, 13, 14), on the immunoprecipitation of cell extracts with anti-idiotypic antibodies against anti-BSA immunoglobulins (12) or on SDS-PAGE of extracts of cells coupled with radioiodinated BSA (11). In contrast, only a glycoprotein of 60 kDa has been detected on affinity chromatography with the use of lectin- or albumin-modified gel (15-18). The reason for the detection of no ABPs other than the 18-kDa peptide with our assay may be the use of different cell lines or the application of different experimental methods. Anyhow, it is certain that the 18-kDa peptide detected in both MDA-MB-453 tumor cells and BAEC is the principal ABP on these cells.

As to the binding selectivity of BSA and HSA as to tumor cells, both albumins were able to bind to both human (MDA-MB-453 and K562) and mouse (X63) tumor cell lines (Table II). Since BSA and HSA competed for binding to MDA-MB-453 cells and BAEC, as observed in cross-linking and immunoprecipitation experiments on ABP (Figs. 6 and 7), it is possible that BSA and HSA share the ABP of 18 kDa on these cells. However, mouse Meth-A cells were able to bind a significant amount of HSA but not BSA, indicating that Meth-A cells may express other ABP(s) which show high affinity for HSA but very low or no affinity for BSA.

ABP are supposed to play a significant role in the transcytosis process. Serum albumin was found to bind specifically with the corresponding binding protein and to cross the endothelial barrier to enter the interstitial fluid (1, 2, 4-6). Although we found that tumor cells also bind serum albumin on their surface with the aid of the 18-kDa ABP, the peptide sequence, the gene encoding the peptide and the physiological role of the ABP in tumor cells remain to be determined.

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