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Detection of Altered Adhesion Molecule Expression in Experimental Tumors by a Radiolabeled Monoclonal Antibody

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Adhesion molecules play a major role in the processes of invasion and metastasis of malignant tumors. Their expression within tumors has been reported to be quantitatively and qualitatively altered according to the invasiveness and metastatic potential of the tumor. The present study tested whether the intratumoral expression of integrin $\alpha 3$ can be detected by a radiolabeled monoclonal antibody. The *in vitro* binding study with four different human cancer cells showed that radioiodinated GA17 antibody recognizing integrin $\alpha 3$ bound specifically to these cells to varying degrees, according to the antigen density on each cell. The biodistribution study with ^{125}I- and ^{111}In-labeled antibodies showed specific localization of radiolabeled GA17 to the xenografts. However, the *in vivo* tumor localization was not proportional to the antigen density calculated *in vitro*, and antibody metabolism varied among the tumors, as was also confirmed by *in vitro* radionuclide retention assay. The intratumoral distribution of radioactivities varied reflecting the antigen expression within the tumor. These results indicate that 1) integrin $\alpha 3$ was expressed in various kinds of tumors and could be localized by the radiolabeled antibody, and 2) the expression of integrin $\alpha 3$ and the metabolism of the radiolabeled antibody after binding to the antigen within the tumor were variable among the tumors, which affected the radionuclide distribution characteristics. The expression of adhesion molecules within these tumors was noninvasively detected by a radiolabeled antibody. It may be possible to use integrin $\alpha 3$, when it is overexpressed, as a target of therapy with antibodies radiolabeled with α or β emitters.

Key words: Adhesion molecule — Integrin $\alpha 3$ — Monoclonal antibody — Radioimmunodetection — Experimental tumor

Despite advances in cancer therapy, local invasion and distant metastasis continue to be difficult to cure and are often fatal.[1, 2] Cancer invasion and metastasis are multistep phenomena involving neoangiogenesis, and complex interactions between cancer cells and the extracellular matrix, vascular endothelium, and the secondary target site.[3–5] Recent investigations indicate that adhesion molecules play a major role in these interactions and are critical factors in invasion and metastasis.[6, 7] Immunohistochemical studies of surgical specimens have shown that the intratumoral expression of adhesion molecules is qualitatively and quantitatively altered according to the malignant potential, invasiveness, and metastatic ability of the cancer.[8–14] It is therefore of clinical importance to determine the intratumoral expression of various adhesion molecules related to tumor invasion and metastasis. This may be possible by biopsy before surgery, and/or by exploration of the surgical specimen after surgery. Biopsy specimens, however, are sometimes not informative or may give false results. A noninvasive evaluation of the expression of adhesion molecules at the beginning of therapy may provide useful information about the invasiveness and metastatic potential of the tumor, contributing to the choice of the appropriate therapeutic modality.

Integrin $\alpha 3$ forms a heterodimer with integrin $\beta 1$ to construct a receptor for collagen, fibronectin, and laminin called VLA-3.[15, 16] It is reported that VLA-3 is expressed in a limited number of normal tissues, but is almost always expressed in plastic-adherent tumor cell lines.[8, 17–20] In the present study, we tested whether the intratumoral expression of integrin $\alpha 3$, which is related to the progression of malignant melanoma,[9, 10] can be noninvasively detected by means of a radiolabeled mAb.

MATERIALS AND METHODS

Human cancer cell lines The human glioblastoma cell line U87MG, the human colon adenocarcinoma cell line LS180 (both obtained from American Type Culture Collection, Rockville, MD), and the human squamous cell carcinoma cell line AOI (kindly supplied by Dr. M. Akiyama, Radiation Research Foundation, Hiroshima) were grown in RPMI1640 medium (Nissui Pharmaceu-

The abbreviations used are: DTPA, diethylenetriaminepentaacetic acid; PBS, phosphate-buffered saline; TCA, trichloroacetic acid; mAb, monoclonal antibody; FCS, fetal calf serum.

tical Co., Tokyo) supplemented with 10% fetal calf serum (Gibco Laboratories, Grand Island, NY) and 0.03% L-glutamine. The human malignant melanoma cell line MeWo (kindly supplied by the Japanese Cancer Research Resources Bank, Tokyo) was grown in Eagle's minimal essential medium (Nissui) also supplemented with 10% fetal calf serum and 0.03% L-glutamine.

mAbs and radiolabeling The murine IgG_1 mAb GA17 was originally produced as a glioma-specific antibody by immunizing mice with human gliosarcoma cells,[21] but later turned out to be specific for human integrin $\alpha 3$; this was proved by amino acid sequence analysis of the affinity-purified protein and also by the reactivity of the immunoprecipitated protein with polyclonal anti-integrin $\alpha 3$ antibody (unpublished observation). The murine IgG_1 mAb 56C, reactive with human chorionic gonadotropin, was used as an isotype-matched control antibody.

Both antibodies were radioiodinated using the chloramine-T method.[22] Purified antibodies (50 μg) in 0.3 *M* phosphate buffer, pH 7.5, and ^{125}I (11.1 MBq) for protein labeling (DuPont/NEN, Wilmington, DE) were mixed with 2.5 μg of chloramine-T (Nakalai Tesque, Inc., Kyoto). After 5 min of reaction, radiolabeled antibodies were separated from free iodine through PD-10 gel chromatography (Pharmacia LKB Biotechnology, Uppsala, Sweden). The specific activities of ^{125}I-labeled antibodies ranged from 101 to 159 MBq/mg for GA17 and from 115 to 152 MBq/mg for 56C.

The two antibodies were labeled with ^{111}In using DTPA as a bifunctional chelating agent.[23] Antibody solution in 0.1 *M* $NaHCO_3$ was mixed with cyclic DTPA anhydride at a DTPA: antibody molar ratio of 3 to 5 for 1 h at room temperature, and then unconjugated DTPA was separated by PD-10 gel chromatography using 0.2 *M* citrate buffer as an eluent. DTPA-conjugated antibody was mixed with ^{111}In-chloride and allowed to stand for 1 h at room temperature. The mixture was then subjected to PD-10 gel chromatography, and free ^{111}In was removed. The labeling efficiency ranged from 70 to 90% for both antibodies. The conjugation ratios of DTPA to GA17 and 56C, estimated as previously described,[23] were 1.68 and 0.86, respectively. Radioiodinated and ^{111}In-labeled antibodies were analyzed by size exclusion high-performance liquid chromatography on a TSKG3000SW column (Tosoh Co., Tokyo). More than 90% of the radioactivity was associated with the IgG fraction. High-molecular weight species, indicating the presence of aggregates, were negligible. Immunoreactivities of radiolabeled GA17 were measured according to the method of Lindmo *et al.*[24] and were more than 80% for both ^{125}I- and ^{111}In-labeled GA17.

***In vitro* binding studies** ^{125}I-labeled antibodies (60,000 cpm/100 μl) were incubated with increasing concentrations of various cancer cells (1×10^5 to 5×10^6) in 5.7- × 46-mm microcentrifuge tubes for 1 h at 4°C. After centrifugation at 10,000*g*, the supernatant was aspirated and the tubes were cut. The radioactivity bound to cells was counted in an auto-well γ-counter. Specific binding was determined by subtracting the binding of ^{125}I-labeled 56C from that of ^{125}I-labeled GA17 at the same cell concentration. To calculate the binding affinity constants and antigen densities on cells using a Scatchard plot analysis,[25] ^{125}I-labeled GA17 and increasing amounts of unlabeled GA17 were incubated with 1 to 2×10^6 cultured cells for 1 h at 4°C, and the radioactivity bound to the cells was counted.

Radionuclide retention assay To examine the fate of radiolabeled antibody after binding, radionuclide retention assay was done according to the method of Naruki *et al.*[26] Forty million cancer cells were incubated with 0.5 μg of ^{125}I-labeled GA17 in 1 ml of RPMI1640 medium with 10% FCS for 1 h at room temperature. After washing, unbound radioactivity was removed and cells were resuspended in individual tubes at a concentration of 2×10^6 cells/ 1 ml of RPMI1640 with 10% FCS. Tubes were incubated at 4°C or 37°C. The cells were separated from the supernatant by centrifugation immediately prior to incubation or after 1, 3, 6, or 20 h of incubation. The cell pellets were washed with cold PBS and the pellets were counted for radioactivity. Supernatants were mixed with 1 ml of 10% trichloroacetic acid and divided into TCA-precipitable and TCA-nonprecipitable fractions. Both fractions were also counted along with the cell pellets. Data were expressed as a percentage of the radioactivity in each fraction.

***In vivo* biodistribution study** Four to 6 million cultured cancer cells were subcutaneously injected into the left flank of female BALB/c nude mice. After 2 to 4 weeks, depending on the cell lines, tumors of about 500-mg weight were formed. Established tumor xenografts were maintained by serial subcutaneous inoculations of tumor pieces.

First, tumor-bearing nude mice were given injections into the tail vein of 37 kBq of ^{125}I-labeled GA17 or 56C. The protein dose was adjusted to 10 μg per mouse by the addition of corresponding unlabeled antibody. At 48 h after injection, groups of mice were killed by ether inhalation, organs were removed and weighed, and the radioactivity was counted. Data were expressed both as percentages of injected dose per gram of tissue normalized to a 20-g mouse and as tumor-to-normal tissue ratios. Localization indices were also obtained, which were derived from the tumor-to-blood ratios of the specific antibody divided by those of the control antibody.

The second experiment was done using both radioiodinated and ^{111}In-labeled antibodies. Nude mice bearing tumor xenografts were injected intravenously with both ^{125}I- and ^{111}In-labeled GA17 or 56C (37 kBq of ^{125}I

and ^{111}In with the protein dose of 10 μg). Groups of mice were killed 48 h later and the biodistributions of ^{125}I- and ^{111}In-labeled antibodies were determined as in the first experiment.

All animal experiments were carried out in accordance with the Japanese regulations regarding animal care and handling. Statistical analysis of the data was done by using Student's *t* test or Welch's test.

Autoradiography The spatial distribution of radioactivities within the tumor was determined by *in vivo* autoradiography. Nude mice bearing human cancer xenografts received an injection into the tail vein of either ^{125}I- or ^{111}In-labeled GA17 (370 kBq/10 μg). Two days later, mice were killed and the tumors were excised and quickly frozen in Tissue-Tek OCT compound (Miles Inc., Elkhart, IN). Serial 10-μm sections were cut from the frozen tissue using a cryomicrotome, and dried well. Sections were placed in a light-tight cassette in direct contact with X-OMAT AR film (Eastman Kodak, Rochester, NY). After 3 to 7 days of exposure, depending on the estimated tumor uptake, the film was processed through an automatic developer. The same or contiguous sections were stained with hematoxylin (Sigma Diagnostics, St. Louis, MO) and eosin (Sigma Diagnostics) for histological reference.

Immunohistochemical staining The expression of integrin $\alpha 3$ in xenografted tumors was immunohistochemically investigated using biotinylated GA17. Briefly, sections were fixed with acetone, and incubated with blocking serum, then with biotinylated GA17 (20 μg/ml) for 1 h at room temperature. They were washed in PBS and incubated with horseradish peroxidase-conjugated streptavidin (Dako Japan, Kyoto). Finally, color was developed by incubation with diaminobenzidine and sections were counterstained with hematoxylin.

RESULTS

***In vitro* binding properties** The *in vitro* binding study showed that ^{125}I-labeled GA17 specifically bound to all four of the human cancer cell lines used (Fig. 1). However, the binding properties varied among the cell lines; the highest binding was seen with U87MG and AOI, and the binding was intermediate with LS180 and low with MeWo. The Scatchard plot analysis showed that the binding affinities did not vary much, ranging from 1.09 to 1.62×10^8 M^{-1}. However, a marked difference was seen among the antigen densities. The antigen density of U87MG, which showed high *in vitro* binding, was 17.4 times higher than that of MeWo, which showed the lowest binding (Table I).

Comparison of radionuclide retention Results of the radionuclide retention assay for U87MG and LS180 cells are summarized in Fig. 2. At 37°C, U87MG showed active release of radioactivity from the cells with a gradual increase of TCA-nonprecipitable radioactivity (probably free iodine), while at 4°C, no significant change in any fraction was observed. LS180 cells, when compared with U87MG cells, showed a very slow release of radioactivity from the cells at 37°C and 87% of the radioactivity was cell-associated even after 20 h of incubation.

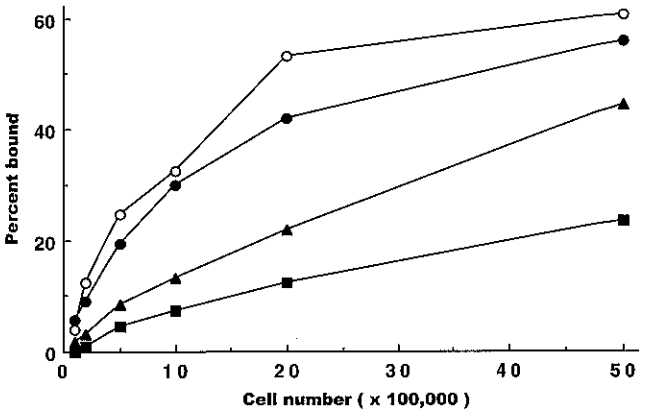

Fig. 1. Cell binding curves of ^{125}I-labeled GA17. ^{125}I-labeled GA17 was incubated with increasing concentrations of human cancer cells. ▲, LS180; ○, AOI; ●, U87MG; ■, MeWo; for 1 h at 4°C. After centrifugation, cell-bound radioactivity was counted. The nonspecific binding of ^{125}I-labeled 56C was subtracted from the binding of ^{125}I-labeled GA17.

Table I. Affinity Constants and Binding Sites per Cell

	Ka (×108)	Binding sites (×105)
U87MG	1.20	8.01
AOI	1.62	6.55
LS180	1.24	1.04
MeWo	1.09	0.46

Biodistribution of ^{125}I-labeled antibodies The biodistribution data of ^{125}I-labeled antibodies in nude mice bearing human cancer xenografts are summarized in Table II. The ^{125}I-labeled GA17 was cleared faster than ^{125}I-labeled 56C from the blood and normal organs. In contrast, the uptake of ^{125}I-labeled GA17 by tumors other than the AOI xenograft was higher than that of ^{125}I-labeled 56C (11.1% vs. 9.0% in U87MG, 17.8% vs. 5.89% in LS180, and 6.48% vs. 2.74% in MeWo; statistically significant in the latter two tumors). As a result, GA17 showed higher tumor-to-normal tissue ratios than did 56C, and the localization indices ranged from 2.0 to 4.4. In the nude mice bearing the AOI xenograft, however, the difference of biodistribution between ^{125}I-labeled GA17 and 56C

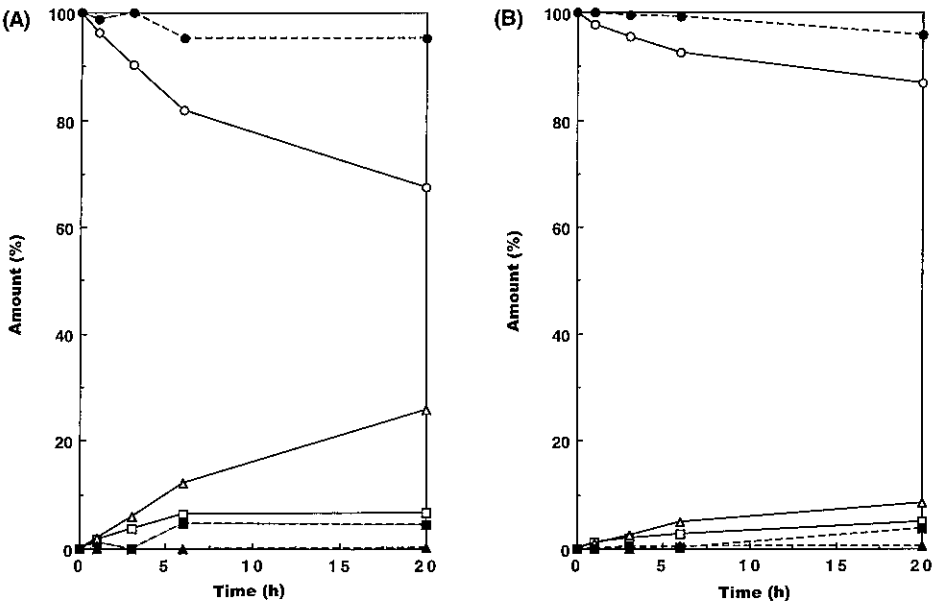

Fig. 2. Comparison of radionuclide retention in U87MG (A) and LS180 (B) cells at 37°C (○ □ △) and 4°C (● ■ ▲). ○ ●, cell-related radioactivity; □ ■, TCA-precipitable radioactivity; △ ▲, TCA-nonprecipitable radioactivity.

was marginal; the tumor-to-normal tissue ratios were not significantly higher with GA17 than with 56C and the localization index was only 1.24.

Comparison of ^{125}I- and ^{111}In-labeled antibodies When ^{111}In-labeled GA17 and ^{125}I-labeled GA17 were coinjected into the same nude mice, these two radiolabels showed different patterns of biodistribution, as shown in Table III. In all tumor-bearing mice, ^{111}In-labeled GA17 showed increased retention in the liver, kidney, and bone. This was also true for radiolabeled 56C (data not shown) and the above biodistribution properties are common for antibodies labeled with ^{111}In by using DTPA as a bifunctional chelate, irrespective of antigen specificity.[27] In addition to this finding, the most striking difference was the significantly increased tumor uptake of ^{111}In-labeled GA17 seen in the three tumors other than LS180. In nude nice bearing U87MG, MeWo, and AOI xenografts, the tumor uptake of ^{111}In-labeled GA17 was 2.28, 2.42, and 1.98 times higher than that of ^{125}I-labeled GA17. The tumor-to-blood ratios were significantly higher with ^{111}In-labeled GA17 than with ^{125}I-labeled GA17 (5.41 vs. 1.59 in U87MG, 3.27 vs. 1.02 in MeWo, 1.58 vs. 0.54 in AOI). In contrast, the tumor uptakes of ^{111}In- and ^{125}I-labeled GA17 were almost identical in nude mice bearing LS180 xenografts.

Spatial distribution of radiolabeled GA17 within the tumor The intratumoral spatial distributions of ^{125}I- and ^{111}In-labeled GA17 were investigated by means of *in vivo* autoradiography. As shown in Fig. 3, the distribution of ^{125}I within the tumor varied among the four human cancer xenografts. In the U87MG xenografts, the distribution of radioactivity was fairly homogeneous except in the areas of necrosis and cystic degeneration, confirmed by hematoxylin and eosin staining of the same section. In the remaining three types of tumors, the radioactivity distributions were quite heterogeneous, even within the viable portion of the tumors. This pattern of intratumoral distribution of the radiolabel was not affected by replacing ^{125}I with ^{111}In as a radiolabel (data not shown).

Antigen expression within the tumor Fig. 4 illustrates the *in vivo* expression of integrin $\alpha 3$ within the tumor xenografts detected by biotinylated GA17. Just as in the distribution pattern revealed by autoradiography (Fig. 3), U87MG showed diffuse and homogeneous antigen expression, whereas the remaining three tumors showed marked heterogeneity in antigen expression; LS180 showed scattered nests of antigen-positive cells, MeWo also showed heterogeneously distributed, weakly antigen-positive areas, and AOI showed a rather diffuse pattern of antigen expression, but was quite heterogeneous in intensity.

DISCUSSION

Recent progress in basic and clinical oncology has shown that adhesion molecules play a major role in the progression of cancer, especially in the processes of invasion and metastasis.[6] Integrins form a major family of

Table II. Biodistribution of ^{125}I-labeled GA17 and 56C in Nude Mice Bearing Human Cancer Xenografts 48 h after Injection of the Antibody

	U87MG		LS180		MeWo		AOI	
	GA17	56C	GA17	56C	GA17	56C	GA17	56C
%ID/gram tissue (normalized to 20-g mouse)								
Blood	5.87*	9.21	9.07*	12.07	6.08**	11.22	8.40*	11.57
	(2.23)	(1.05)	(0.97)	(1.75)	(2.35)	(1.30)	(2.26)	(0.33)
Liver	1.89	3.17	2.67	4.56	1.66**	3.25	2.47*	4.04
	(0.88)	(1.23)	(0.49)	(2.23)	(0.74)	(0.45)	(0.83)	(0.71)
Kidney	1.99*	3.10	2.71**	4.10	1.93**	3.52	2.46	3.27
	(0.58)	(0.42)	(0.37)	(0.68)	(0.76)	(0.37)	(0.79)	(0.22)
Lung	1.99*	3.43	3.50*	5.08	2.50**	4.89	3.49*	5.62
	(0.64)	(0.53)	(0.50)	(1.16)	(1.00)	(0.91)	(0.97)	(0.37)
Muscle	0.73*	1.05	0.89*	1.14	0.54**	0.99	0.86	1.11
	(0.27)	(0.08)	(0.16)	(0.06)	(0.19)	(0.03)	(0.31)	(0.12)
Bone	0.83	1.45	0.99*	1.95	0.77**	1.41	1.35	1.86
	(0.33)	(0.83)	(0.16)	(0.68)	(0.28)	(0.13)	(0.47)	(0.14)
Tumor	11.01	9.00	17.80**	5.89	6.48*	2.74	4.73	5.32
	(3.12)	(1.69)	(1.13)	(1.55)	(2.24)	(0.20)	(1.51)	(1.52)
Tumor-to-normal tissue ratios and localization indices								
T/Blood	1.94**	0.97	1.98**	0.49	1.10**	0.25	0.57	0.46
	(0.27)	(0.11)	(0.25)	(0.10)	(0.23)	(0.02)	(0.12)	(0.12)
T/Liver	6.38**	2.91	6.82**	1.42	4.16**	0.85	2.01	1.32
	(1.80)	(0.55)	(1.17)	(0.47)	(1.21)	(0.07)	(0.55)	(0.26)
T/Kidney	5.54**	2.89	6.64**	1.42	3.48**	0.78	2.02	1.62
	(0.25)	(0.21)	(0.67)	(0.14)	(0.78)	(0.03)	(0.55)	(0.39)
T/Lung	5.57**	2.61	5.14**	1.16	2.69**	0.57	1.40	0.94
	(0.53)	(0.10)	(0.55)	(0.14)	(0.64)	(0.08)	(0.45)	(0.22)
T/Muscle	15.56**	8.54	20.52**	5.14	12.07**	2.77	5.86	4.72
	(1.90)	(1.54)	(4.16)	(1.16)	(2.41)	(0.25)	(1.68)	(0.82)
T/Bone	13.79**	6.44	18.35**	3.08	8.68**	1.94	3.68	2.87
	(2.37)	(1.39)	(2.49)	(0.28)	(2.35)	(0.09)	(0.99)	(0.85)
LI	2.00		4.04		4.40		1.24	

Mean (SD) of 4 to 6 mice.
* $P<0.05$ compared with 56C. ** $P<0.01$ compared with 56C.
T: tumor. LI: localization index.

adhesion molecules and their role in cancer progression has been investigated. In the present investigation, we tested whether the intratumoral expression of integrin $\alpha 3$ can be detected by radiolabeled mAbs.

The murine mAb designated GA17 was originally developed as a human malignant glioma-specific antibody,[21] but it later showed wide reactivity with other cancer cells.[28] The present study also demonstrated that radiolabeled GA17 specifically binds to human colon adenocarcinoma, human lung squamous cell carcinoma, and human malignant melanoma cell lines, in addition to a human malignant glioma cell line. This wide spectrum of reactivity is not surprising since GA17 turned out to be an antibody against integrin $\alpha 3$. The binding affinity of GA17 to integrin $\alpha 3$ on the four cell lines examined in the present study did not vary greatly, and the density of integrin molecules on the cell was the major determinant of their differential *in vitro* binding.

Our *in vivo* experiment showed that radiolabeled GA17 specifically localized to the tumor. However, the tumor-localizing ability varied among the four different cancer xenografts, and also between ^{125}I- and ^{111}In-labeled GA17. First, in three cancer xenografts, U87MG, MeWo, and AOI, ^{111}In-labeled GA17 showed a markedly increased tumor uptake when compared to ^{125}I-labeled GA17. This discrepant tumor uptake between ^{125}I- and ^{111}In-labeled antibody strongly suggests that the antibody-antigen complexes formed on the cancer cell surface were internalized and actively metabolized, and the free iodine formed by dehalogenation escaped rapidly from the cancer cells, while ^{111}In remained inside the cells.[26,27] This seems likely, considering the function of integrin as a signaling molecule in cell-cell or cell-extracellular interaction. The tumor uptakes of ^{125}I- and ^{111}In-labeled GA17 were almost identical in the LS180 xenograft, indicating that in this tumor, the metabolism of the

Table III. Biodistribution of ^{125}I- and ^{111}In-labeled GA17 in Nude Mice Bearing Human Cancer Xenografts 48 h after Injection of the Antibody

	U87MG		LS180		MeWo		AOI	
	In-111	I-125	In-111	I-125	In-111	I-125	In-111	I-125
%ID/gram tissue (normalized to 20-g mouse)								
Blood	3.47*	5.05	5.35	7.16	5.37	7.07	6.30	9.36
	(0.61)	(1.12)	(1.52)	(2.61)	(1.77)	(3.12)	(2.30)	(4.51)
Liver	9.86**	1.60	11.01**	1.93	10.08**	1.87	11.48**	3.11
	(1.34)	(0.32)	(1.10)	(0.92)	(0.78)	(1.27)	(0.78)	(1.82)
Kidney	20.50**	1.60	19.07**	2.11	17.21**	2.09	20.13**	2.81
	(5.56)	(0.46)	(1.31)	(0.57)	(0.65)	(0.77)	(1.65)	(1.26)
Lung	2.77**	2.00	3.12	3.04	4.63	3.95	3.62	3.75
	(0.24)	(0.20)	(0.86)	(1.33)	(2.46)	(1.56)	(0.76)	(1.72)
Muscle	1.07*	0.68	0.80	0.60	0.88	0.63	1.01	0.76
	(0.27)	(0.27)	(0.14)	(0.22)	(0.15)	(0.27)	(0.03)	(0.29)
Bone	2.22**	0.72	2.75**	0.84	2.51**	0.84	2.70**	0.94
	(0.40)	(0.18)	(0.39)	(0.35)	(0.12)	(0.29)	(0.54)	(0.38)
Tumor	18.53**	8.14	13.62	12.95	17.07**	7.04	9.17**	4.64
	(3.92)	(4.03)	(1.33)	(2.09)	(3.64)	(2.60)	(1.42)	(1.66)
Tumor-to-normal tissue ratios and localization indices								
T/Blood	5.41**	1.59	2.66*	1.91	3.27**	1.02	1.58**	0.54
	(1.11)	(0.58)	(0.53)	(0.38)	(0.37)	(0.09)	(0.44)	(0.13)
T/Liver	1.89*	4.89	1.25**	7.51	1.71**	4.47	0.80*	1.82
	(0.36)	(1.55)	(0.22)	(2.15)	(0.43)	(1.47)	(0.16)	(0.71)
T/Kidney	0.97**	5.11	0.71**	6.27	0.99**	3.40	0.46**	1.75
	(0.35)	(1.89)	(0.05)	(0.74)	(0.18)	(0.42)	(0.07)	(0.34)
T/Lung	6.75*	4.07	4.54	4.61	4.38*	1.98	2.57**	1.31
	(1.61)	(2.01)	(0.87)	(1.03)	(1.68)	(0.75)	(0.26)	(0.25)
T/Muscle	17.97	12.72	17.23*	22.83	19.40**	11.46	9.05**	6.23
	(4.91)	(6.23)	(1.80)	(4.68)	(3.41)	(1.13)	(1.23)	(0.88)
T/Bone	8.36	10.92	4.99**	17.02	6.78	8.46	3.46*	5.15
	(1.04)	(3.41)	(0.54)	(5.06)	(1.29)	(1.17)	(0.62)	(1.17)
LI	4.13	2.21	3.24	4.15	4.04	4.43	2.08	1.54

Mean (SD) of 5 mice.
* $P<0.05$ compared with I-125 labeled GA17. ** $P<0.01$ compared with I-125 labeled GA17.
T: tumor. LI: localization index.

antibody was not so active, which might be a reflection of different functioning of the antigen. This heterogeneity in antibody metabolism was also confirmed *in vitro* by radionuclide retention assay of U87MG and LS180 cells. U87MG cells, which exhibited discrepant *in vivo* tumor localization between ^{125}I- and ^{111}In-labeled GA17, showed relatively active release of radioactivity from the cells with time when incubated at 37°C. On the other hand, LS180 cells, which showed identical tumor localization of ^{125}I- and ^{111}In-labeled GA17, showed inactive metabolism when incubated at 37°C.

^{125}I-labeled GA17 was most markedly accumulated in the LS180 xenografts, followed by U87MG, MeWo, and AOI. This order of tumor accumulation is different from the order of *in vitro* binding at 4°C, i.e., antigen density. This is not surprising when we consider the presence of active antibody internalization and metabolism in the three tumors other than LS180. However, the order of tumor uptake of ^{111}In-labeled GA17 (U87MG>MeWo >LS180>AOI), which is known to be unaffected by the presence of active metabolism of the antibody, was also not proportional to *in vitro* binding, indicating that other factors than the difference in antibody metabolism and antigen density also affect the tumor localization.

Furthermore, the intratumoral distribution of radiolabeled GA17 detected by autoradiography also suggested a heterogeneous nature of these *in vivo* tumors. In three of the four tumor xenografts, the distribution of radioactivity was quite heterogeneous, while in the remaining glioma xenograft, it was fairly homogeneous. Since various factors affect the intratumoral distribution of radiolabeled antibodies[29–32] and the present experiment was done with only one dose and at one time point, these data are insufficient to allow a conclusion to be reached. However, immunohistochemical staining showed heterogeneous antigen expression within the

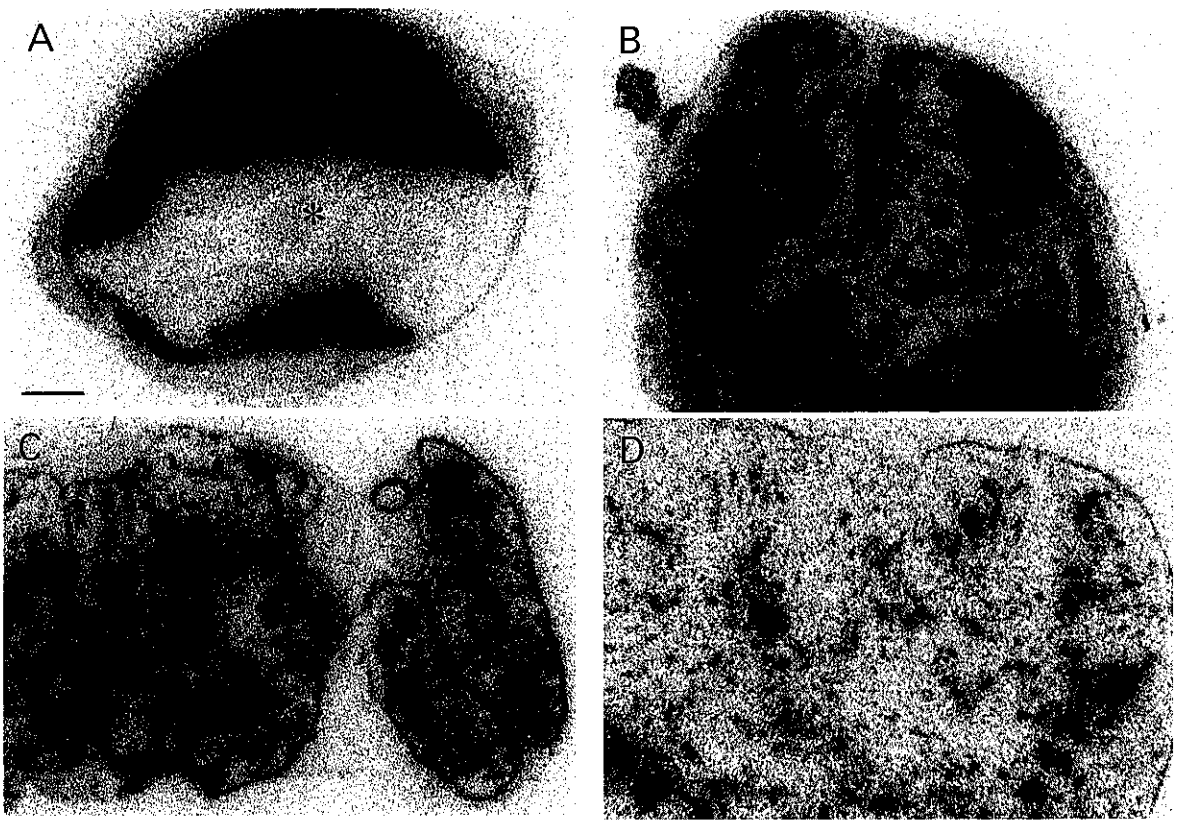

Fig. 3. Spatial distribution of ^{125}I-labeled GA17 in four tumor xenografts detected by autoradiography. A, U87MG; B, LS180; C, MeWo; D, AOI. The asterisk in A represents the necrotic and cystic area confirmed by HE staining. Original magnification $\times 3.3$. The bar indicates 1 mm.

three tumors that showed heterogeneous radioactivity distribution, and fairly homogeneous antigen expression in the remaining U87MG tumor. Therefore, we can at least say that the expression of integrin $\alpha 3$ *in vivo* is partially reflected in the intratumoral distribution of radiolabeled antibody.

The present investigation showed that integrin $\alpha 3$, which constructs VLA-3 with integrin $\beta 1$, is widely expressed in various cancer cell lines *in vitro* and can be localized *in vivo* by radiolabeled GA17 antibody. The role of VLA-3 in tumorigenesis and tumor progression has not been fully investigated and is controversial. The expression of VLA-3 is reported to be quantitatively and qualitatively altered in various cancers. The progression of malignant melanoma and some other cancers is related to VLA-3 expression,[9, 10, 33, 34] but in some other cancers, the VLA-3 expression is downregulated.[11, 14, 35, 36] In the present study, there was a significant change in integrin $\alpha 3$ expression in various cancer cell lines from *in vitro* to *in vivo*, and the fate of a specific antibody was different among the tumor types, probably reflecting the heterogeneous *in vivo* nature of VLA-3 expression and function. Although tumor uptake of a radiolabeled specific antibody was affected by factors other than antigen expression, and the exact quantitation of integrin expression was impossible, altered intratumoral expression of VLA-3 determined at least partially the intratumoral distribution of radiolabeled GA17. We need to consider that the results of studies with established cell lines, which are highly selected populations, may not be easily extrapolated to the clinical situation, and that the expression of adhesion molecules is a dynamic phenomenon and can change according to the status of the cancer (early vs. advanced stage). The present results warrant further study in a more clinically relevant setting, such as an orthotopic transplant model that shows local invasion and metastasis. Another point to consider is the presence of normally expressed integrin $\alpha 3$. For the antigen to work as the target of radioimmunodetection and radioimmunotherapy, the antigen expression should ideally be localized to the tumors. In the case of integrin, which is a functional molecule expressed in some normal tissues,

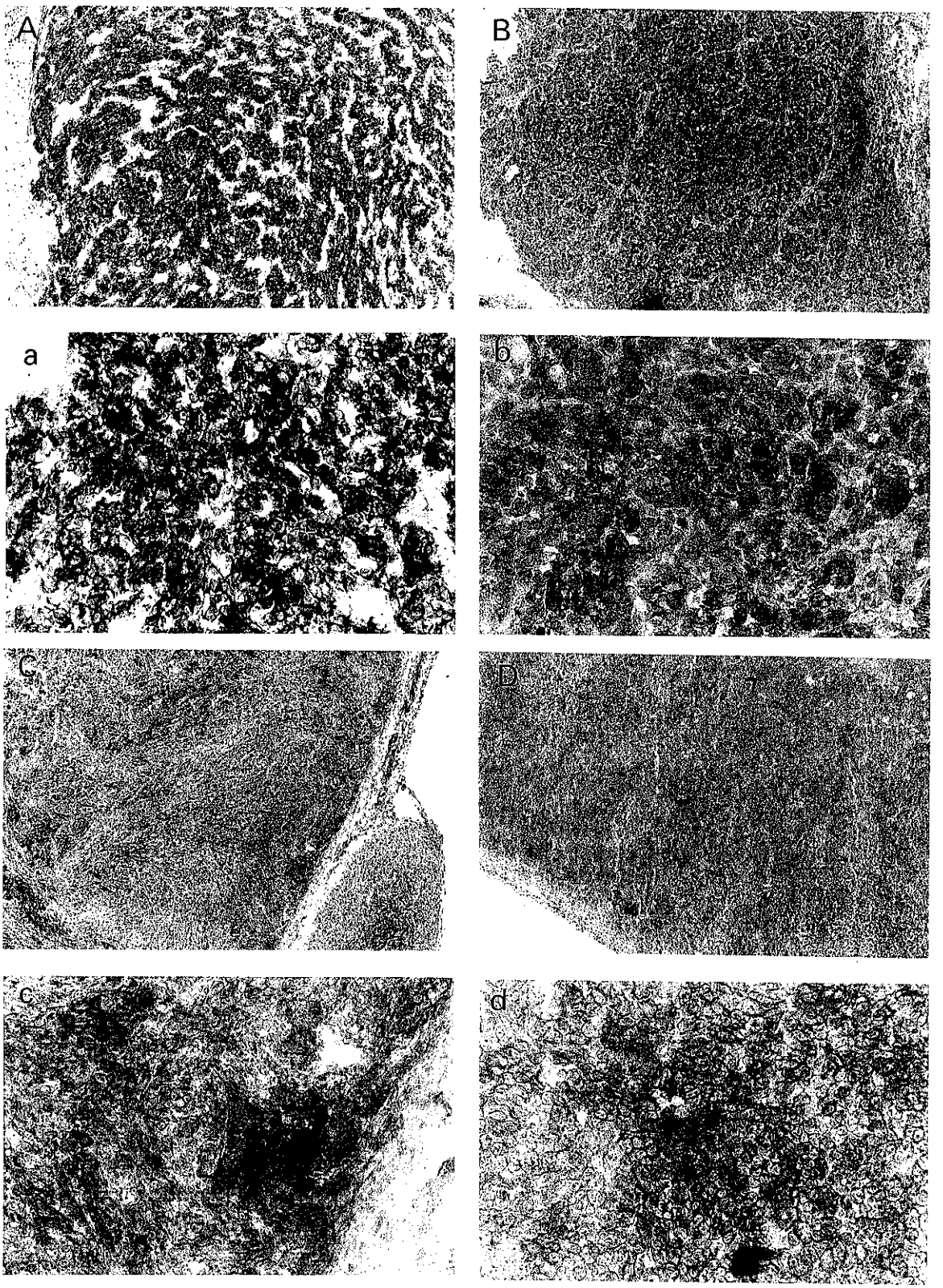

Fig. 4. Immunohistochemical staining of tissue sections made from four tumor xenografts. A, a, U87MG; B, b, LS180; C, c, MeWo; D, d, AOI. Original magnification: A, B, C, D, ×16; a, b, c, d, ×80.

the specificity ratio might be low, but the quantitative difference (higher antigen density in cancer cells than normal cells) and qualitative difference (loss of polarity of antigen expression in cancer cells may make the antigen in the tumor more accessible to blood-borne antibody) may make it a favorable target of antibody-based imaging and therapy.

In conclusion, the tumoral integrin $\alpha 3$, which shows qualitatively and quantitatively altered *in vivo* expression, can be detected by radiolabeled GA17, and when it is overexpressed, it may be useful as a target of radiolabeled antibody-mediated imaging and therapy. Further investigation of other adhesion molecules which may be more closely related to cancer progression and neoangiogenesis, such as VLA-6, integrin $\alpha v/\beta 3$, and non-integrin laminin receptors,[13, 37–39] is also necessary.

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REFERENCES

1) Fidler, I. J. and Hanna, M. G., Jr. New approaches to specific and non-specific immunotherapy of established cancer metastases. *In* "Fundamental Mechanisms in Human Cancer Immunology," ed. J. P. Saunders, pp. 425–437 (1981). Elsevier, North Holland.
2) Liotta, L. A. Cancer cell invasion and metastasis. *Sci. Am.*, Feb., 54–63 (1992).
3) Liotta, L. A. Gene products which play a role in cancer invasion and metastasis. *Breast Cancer Res. Treat.*, **11**, 113–124 (1988).
4) Folkman, J. and Shing, Y. Angiogenesis. *J. Biol. Chem.*, **267**, 10931–10934 (1992).
5) Hart, I. R. and Saini, A. Biology of tumor metastasis. *Lancet*, **339**, 1453–1457 (1992).
6) Albelda, S. M. Biology of disease: role of integrins and other cell adhesion molecules in tumor progression and metastasis. *Lab. Invest.*, **68**, 4–17 (1993).
7) Schwartz, M. A. Signaling by integrins: implication for tumorigenesis. *Cancer Res.*, **53**, 1503–1506 (1993).
8) Bartolazzi, A., Cerboni, C., Full, C., Valentini, C., Natali, P. G., Venturo, I. and Bigotti, A. VLA-3 distribution in normal and neoplastic non-lymphoid human tissues. *Pathol. Res. Pract.*, **189**, 387–393 (1993).
9) Natali, P. G., Nicotra, M. R., Di Filippo, F. and Bigotti, A. Expression of fibronectin, fibronectin isoforms and integrin receptors in melanocytic lesions. *Br. J. Cancer*, **71**, 1243–1247 (1995).
10) Natali, P. G., Nicotra, M. R., Bartolazzi, A., Cavaliere, R. and Bigotti, A. Integrin expression in cutaneous malignant melanoma: association of the $\alpha 3/\beta 1$ heterodimer with tumor progression. *Int. J. Cancer*, **54**, 68–72 (1993).
11) Liebert, M., Washington, R., Stein, J., Wedemeyer, G. and Grossman, H. B. Expression of the VLA $\beta 1$ integrin family in bladder cancer. *Am. J. Pathol.*, **144**, 1016–1022 (1994).
12) Albelda, S. M., Mette, S. A., Elder, D. E., Stewart, R., Damjanovich, L., Herlyn, M. and Buck, C. A. Integrin distribution in malignant melanoma: association of the $\beta 3$ subunit with tumor progression. *Cancer Res.*, **50**, 6757–6764 (1990).
13) Friedrichs, K., Ruiz, P., Franke, F., Gille, I., Terpe, H.-J. and Imhof, B. A. High expression level of $\alpha 6$ integrin in human breast cancer is correlated with reduced survival. *Cancer Res.*, **55**, 901–906 (1995).
14) Gui, G. P., Wells, C. A., Browne, P. D., Yeomans, P., Jordan, S., Puddefoot, J. R., Vinson, G. P. and Carpenter, R. Integrin expression in primary breast cancer and its relation to axillary nodal status. *Surgery*, **117**, 102–108 (1995).
15) Wayner, E. A., Carter, W. G., Piotrowicz, R. S. and Kunicki, T. J. The function of multiple extracellular matrix receptors in mediating cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoproteins Ic-IIa. *J. Cell Biol.*, **107**, 1881–1891 (1988).
16) Takada, Y., Wayner, E. A., Carter, W. G. and Hemler, M. E. Extracellular matrix receptors, ECMRII and ECMRI, for collagen and fibronectin correspond to VLA-2 and VLA-3 in the VLA family of heterodimers. *J. Cell. Biochem.*, **37**, 385–393 (1988).
17) Carter, W. G., Wayner, E. A., Bouchard, T. S. and Kaur, P. The role of integrins $\alpha 2\beta 1$ and $\alpha 3\beta 1$ in cell-cell and cell-substrate adhesion of human epidermal cells. *J. Cell Biol.*, **110**, 1387–1404 (1990).
18) Fradet, Y., Cordon-Cardo, C., Thomson, T., Daly, M. E., Whitmore, W. F., Jr., Lloyd, K. O., Melamed, M. R. and Old, L. J. Cell surface antigens of human bladder cancer defined by mouse monoclonal antibodies. *Proc. Natl. Acad. Sci. USA*, **81**, 224–228 (1984).
19) Morhenn, V. B., Schreiber, A. B., Soriero, O., McMillan, W. and Allison, A. C. A monoclonal antibody against basal cells of human epidermis. *J. Clin. Invest.*, **76**, 1978–1983 (1985).
20) Peltonen, J., Larjava, H., Jaakkola, S., Gralnick, H., Akiyama, S. K., Yamada, S. S., Yamada, K. M. and Uitto,

J. Localization of integrin receptors for fibronectin, collagen, and laminin in human skin. *J. Clin. Invest.*, **84**, 1916–1923 (1989).
21) Kondo, S., Miyatake, S., Iwasaki, K., Oda, Y., Kikuchi, H., Zu, Y., Shamoto, M. and Namba, Y. Human glioma-specific antigens detected by monoclonal antibodies. *Neurosurgery*, **30**, 506–511 (1992).
22) Hunter, W. N. and Greenwood, F. C. Preparation of iodine-131 labeled human growth hormone of high specific activity. *Nature*, **194**, 495–496 (1962).
23) Sakahara, H., Endo, K., Nakashima, T., Koizumi, M., Ohta, H., Torizuka, K., Furukawa, T., Ohmomo, Y., Yokoyama, A., Okada, K., Yoshida, O. and Nishi, S. Effect of DTPA conjugation on the antigen binding activity and biodistribution of monoclonal antibodies against alpha-fetoprotein. *J. Nucl. Med.*, **26**, 750–755 (1985).
24) Lindmo, T., Boven, E., Cuttitta, F., Fedorko, J. and Bunn, P. A., Jr. Determination of the immunoreactive fraction of radiolabeled monoclonal antibody by linear extrapolation to binding at infinite antigen excess. *J. Immunol. Methods*, **72**, 77–89 (1984).
25) Scatchard, G. The attraction of proteins for small molecules and ions. *Ann. NY Acad. Sci.*, **51**, 660–672 (1949).
26) Naruki, Y., Carrasquillo, J. A., Reynolds, J. C., Maloney, P. J., Frincke, J. M., Neumann, R. D. and Larson, S. M. Differential cellular catabolism of ^{111}In, ^{90}Y and ^{125}I radiolabeled T101 anti-CD5 monoclonal antibody. *Nucl. Med. Biol.*, **17**, 201–207 (1990).
27) Saga, T., Endo, K., Akiyama, T., Sakahara, H., Koizumi, M., Watanabe, Y., Nakai, T., Hosono, M., Yamamoto, T., Toyoshima, K. and Konishi, J. Scintigraphic detection of overexpressed c-erbB-2 protooncogene product by a class-switched murine monoclonal anti-c-erbB-2 protein monoclonal antibody. *Cancer Res.*, **51**, 990–994 (1991).
28) Kobayashi, H., Sakahara, H., Hosono, M., Shirato, M., Kondo, S., Miyatake, S., Kikuchi, H., Namba, Y., Endo, K. and Konishi, J. Scintigraphic detection of neural-cell-derived small-cell lung cancer using glioma-specific monoclonal antibody. *J. Cancer Res. Clin. Oncol.*, **120**, 259–262 (1994).
29) Saga, T., Neumann, R. D., Heya, T., Sato, J., Kinuya, S., Le, N., Paik, C. H. and Weinstein, J. N. Targeting cancer micrometastases with monoclonal antibodies: a binding site barrier. *Proc. Natl. Acad. Sci. USA*, **92**, 8999–9003 (1995).
30) Sands, H., Jones, P. L., Shah, S. A., Palme, D., Vessalla, R. L. and Gallagher, B. M. Correlation of vascular permeability and blood flow with monoclonal antibody uptake by human Cloeser and renal cell xenografts. *Cancer Res.*, **48**, 188–193 (1988).
31) Dvorak, H. F., Nagy, J. A., Dvorak, J. T. and Dvorak, A. M. Identification and characterization of the blood vessels of solid tumors that are leaky to circulating macromolecules. *Am. J. Pathol.*, **133**, 95–109 (1988).
32) Jain, R. K. and Baxter, L. T. Mechanism of heterogeneous distribution of monoclonal antibodies and other macromolecules in tumors: significance of elevated interstitial pressure. *Cancer Res.*, **48**, 7022–7032 (1988).
33) Yasoshima, T., Denno, R., Kawaguchi, S., Sato, N., Okada, Y., Ura, H., Kikuchi, K. and Hirata, K. Establishment and characterization of human gastric carcinoma lines with high metastatic potential in the liver: changes in integrin expression associated with the ability to metastasize in the liver of nude mice. *Jpn. J. Cancer Res.*, **87**, 153–160 (1996).
34) Mortarini, R., Anichini, A. and Parmiani, G. Heterogeneity for integrin expression and cytokine-related VLA modulation can influence the adhesion of human melanoma cells to extracellular matrix proteins. *Int. J. Cancer*, **47**, 551–559 (1991).
35) Lessey, B. A., Albelda, S., Buck, C. A., Castelbaum, A. J., Yeh, I., Kohler, M. and Berchuck, A. Distribution of integrin cell adhesion molecules in endometrial cancer. *Am. J. Pathol.*, **146**, 717–726 (1995).
36) Dedhar, S., Saulnier, R., Nagle, R. and Overall, C. M. Specific alterations in the expression of alpha 3 beta 1 and alpha 6 beta 4 integrins in highly invasive and metastatic variants of human prostate carcinoma cells selected by *in vitro* invasion through reconstituted basement membrane. *Clin. Exp. Metastasis*, **11**, 391–400 (1993).
37) Weinel, R. J., Rosendahl, A., Pinschmidt, E., Kisker, O., Simon, B. and Santoso, S. The α6-integrin receptor in pancreatic carcinoma. *Gastroenterology*, **108**, 523–532 (1995).
38) Pellegrini, R., Martignone, S., Menard, S. and Colnaghi, M. I. Laminin receptor expression and function in small-cell lung carcinoma. *Int. J. Cancer*, **8** (Suppl.), 116–120 (1994).
39) Brooks, P. C., Clark, R. A. F. and Cheresh, D. A. Requirement of vascular integrin $\alpha v\beta 3$ for angiogenesis. *Science*, **264**, 569–571 (1994).